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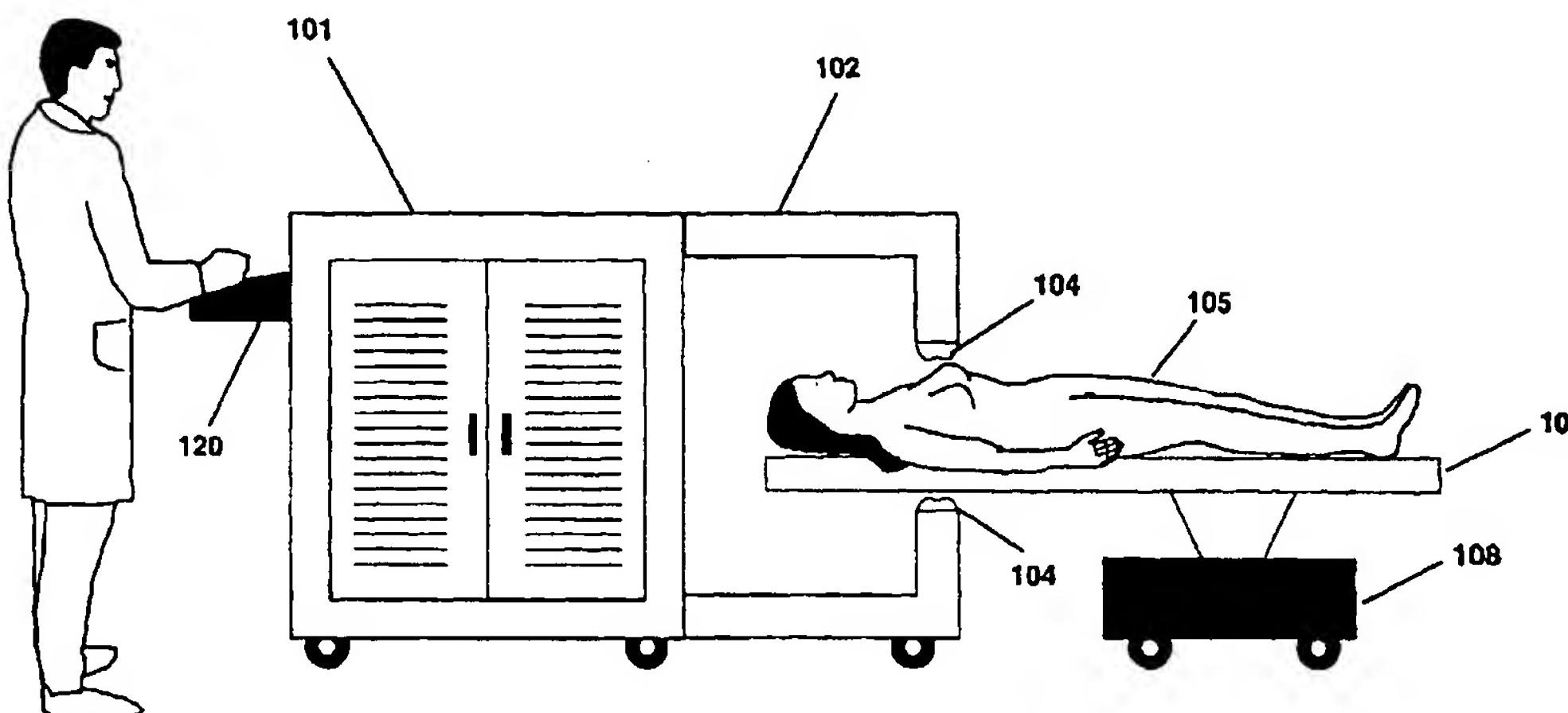
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(54) Title: THERMOTHERAPY VIA TARGETED DELIVERY OF NANOSCALE MAGNETIC PARTICLES



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(57) Abstract: Disclosed are therapeutic methods for the treatment of diseased, disease-causing, or undesirable tissue or material that involve the administration of a thermotherapeutic magnetic composition, which contains single-domain magnetic particles attached to a target-specific ligand, to a patient and the application of an alternating magnetic field to inductively heat the thermotherapeutic magnetic composition. Also disclosed are methods for administration of the thermotherapeutic magnetic composition. These therapeutic methods may be used where the predetermined target is associated with a disease, such as cancer, diseases of the immune system, pathogen-borne diseases, obesity, hormone-related diseases, Alzheimer's disease, disease precursor, and undesirable materials, such as undesirable adipose tissue, reactions to organ transplants, HTV infected cells, polypeptides receptors, blood clots, and low density lipoproteins.



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## **THERMOTHERAPY VIA TARGETED DELIVERY OF NANOSCALE MAGNETIC PARTICLES**

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### **TECHNICAL FIELD**

The present invention relates generally to thermotherapy, more specifically, to magnetic material compositions, devices for use with magnetic material compositions, and methods related thereto for thermotherapy via targeted delivery of nanoscale magnetic particles.

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### **BACKGROUND**

The time between the onset of disease in a patient and the conclusion of a successful course of therapy is often unacceptably long. Many diseases remain asymptomatic and evade detection while progressing to advanced and often terminal stages. In addition, this period may be marked by significant psychological and physical trauma for the patient due to the unpleasant side effects of even correctly prescribed treatments. Even those diseases that are detected early may be most effectively treated only by therapies that disrupt the normal functions of healthy tissue or have other unwanted side effects.

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One such disease is cancer. Despite considerable research effort and some success, cancer is still the second leading cause of death in the United States, claiming more than 500,000 lives each year according to American Cancer Society estimates. Traditional treatments are invasive and/or are attended by harmful side effects (e.g., toxicity to healthy cells), often making for a traumatic course of therapy with only modest success. Early detection, a result of better diagnostic practices and technology, has improved the prognosis for many patients. However, the suffering that many patients must endure makes for a more stressful course of therapy and may complicate patient compliance with prescribed therapies. Further, some cancers defy currently available treatment options, despite improvements in disease detection. Of the many forms of cancer that still pose a medical challenge, prostate,

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breast, lung, and liver claim the vast majority of lives each year. Colorectal cancer, ovarian cancer, gastric cancer, leukemia, lymphoma, melanoma, and their metastases may also be life-threatening.

Conventional treatments for breast cancer, for example, typically include  
5 surgery followed by radiation and/or chemotherapy. These techniques are not always effective, and even if effective, they suffer from certain deficiencies. Surgical procedures range from removal of only the tumor (lumpectomy) to complete removal of the breast. In early stage cancer, complete removal of the breast provides the best assurance against recurrence, but is disfiguring and requires  
10 the patient to make a very difficult choice. Lumpectomy is less disfiguring, but is associated with a greater risk of cancer recurrence. Radiation therapy and chemotherapy are arduous and are not completely effective against recurrence.

Treatment of pathogen-based diseases is also not without complications.  
Patients presenting symptoms of systemic infection are often mistakenly treated with  
15 broad-spectrum antibiotics as a first step. This course of action is completely ineffective when the invading organism is viral. Even if a bacterium (e.g., *E. coli*) is the culprit, the antibiotic therapy eliminates not only the offending bacteria, but also benign intestinal flora in the gut that are necessary for proper digestion of food. Hence, patients treated in this manner often experience gastrointestinal distress until  
20 the benign bacteria can repopulate. In other instances, antibiotic-resistant bacteria may not respond to antibiotic treatment. Therapies for viral diseases often target only the invading viruses themselves. However, the cells that the viruses have invaded and "hijacked" for use in making additional copies of the virus remain viable. Hence, progression of the disease is delayed, rather than halted.

25 For these reasons, it is desirable to provide improved and alternative techniques for treating disease. Such techniques should be less invasive and traumatic to the patient than the present techniques, and should only be effective locally at targeted sites, such as diseased tissue, pathogens, or other undesirable matter in the body. Preferably, the techniques should be capable of being performed  
30 in a single or very few treatment sessions (minimizing the need for patient compliance), with minimal toxicity to the patient. In addition, the undesirable

matter should be targeted by the treatment without requiring significant operator skill and input.

5 Immunotherapy is a rapidly expanding type of therapy used for treating a variety of human diseases including cancer, for example. The FDA has approved a number of antibody-based cancer therapeutics. The ability to engineer antibodies, antibody fragments, and peptides with altered properties (e.g., antigen binding affinity, molecular architecture, specificity, valence, etc.) has enhanced their use in therapies. Cancer immunotherapeutics have made use of advances in the chimerization and humanization of mouse antibodies to reduce immunogenic 10 responses in humans. High affinity human antibodies have also been obtained from transgenic mice that contain many human immunoglobulin genes. In addition, phage display technology, ribosome display, and DNA shuffling have allowed for the discovery of antibody fragments and peptides with high affinity and low 15 immunogenicity for use as targeting ligands. All of these advances have made it possible to design an immunotherapy that has a desired antigen binding affinity and specificity, and minimal immune response.

The field of cancer immunotherapy makes use of markers that are over-expressed by cancer cells (relative to normal cells) or expressed only by cancer cells. The identification of such markers is ongoing and the choice of a ligand/marker 20 combination is critical to the success of any immunotherapy. Immunotherapeutics fall into three classes: (1) deployment of antibodies that, themselves, target growth receptors, disrupt cytokine pathways, or induce complement or antibody-dependent cytotoxicity; (2) direct arming of antibodies with a toxin, a radionuclide, or a cytokine; (3) indirect arming of antibodies by attaching them to immunoliposomes 25 used to deliver a toxin or by attaching them to an immunological cell effector (bispecific antibodies). Although armed antibodies have shown potent tumor activity in clinical trials, they have also exhibited unacceptably high levels of toxicity to patients.

30 The disadvantage of therapies that rely on delivery of immunotoxins or radionuclides (i.e., direct and indirect arming) has been that, once administered to the patient, these agents are active at all times. (A "therapy-on-demand" approach

would be preferable.) These therapies often cause damage to non-tumor cells and present toxicity issues and delivery challenges. For example, cancer cells commonly shed surface-expressed antigens (targeted by immunotherapeutics) into the blood stream. Immune complexes can be formed between the immunotherapeutic and the shed antigen. As a result, many antibody-based therapies are diluted by interaction with these shed antigens instead of interacting with the cancer cells themselves, reducing the true delivered dose.

Temperatures in a range from about 40 °C to about 46 °C (hyperthermia) can cause irreversible damage to diseased cells. However, healthy cells are capable of surviving exposure to temperatures up to around 46.5 °C. Diseased tissue may be treated by elevating the temperature of its individual cells to a lethal level (cellular thermotherapy). Pathogens implicated in disease and other undesirable matter in the body can be also be destroyed via exposure to locally-high temperatures.

Hyperthermia may hold promise as a treatment for cancer because it induces instantaneous necrosis (typically called “thermo-ablation”) and/or a heat-shock response in cells (classical hyperthermia), leading to cell death via a series of biochemical changes within the cell. State-of-the-art systems that employ radio-frequency (RF) hyperthermia, such as annular phased array systems (APAS), attempt to tune E-field energy for regional heating of deep-seated tumors. Such techniques are limited by the heterogeneities of tissue electrical conductivity and that of highly perfused tissue. This leads to the as-yet-unsolved problems of “hot spot” phenomena in untargeted tissue with concomitant underdosage in the desired areas. These factors make selective heating of specific regions with such E-field dominant systems very difficult.

Another strategy that utilizes RF hyperthermia requires surgical implantation of microwave or RF based antennae or self-regulating thermal seeds. In addition to its invasiveness, this approach provides few (if any) options for treatment of metastases because it requires knowledge of the precise location of the primary tumor. The seed implantation strategy is thus incapable of targeting undetected individual cancer cells or cell clusters not immediately adjacent to the primary tumor

site. Clinical success of this strategy is hampered by problems with the targeted generation of heat at the desired tumor tissues.

## SUMMARY OF THE INVENTION

5        Hyperthermia for treatment of disease using magnetic fluids exposed to RF fields has been recognized for several decades. However, a major problem with magnetic fluid hyperthermia has been the inability to selectively deliver a lethal dose of particles to the cells or pathogens of interest.

10      In view of the above, there is a need for a hyperthermia-based treatment method for diseased tissue that incorporates selective delivery of thermotherapeutic magnetic compositions to a diseased tissue. It is also desirable to have methods for treating diseased tissue in a safe and effective manner, with minimal invasion, and short treatment periods.

15      It is therefore an object of the present invention to formulate magnetic material compositions including nanoscale magnetic particles and target-specific ligands useful in the treatment of diseased tissue.

It is another object of the present invention to provide a device for the treatment of diseased tissue that is capable of providing an alternating magnetic field.

20      It is yet another object of the present invention to provide a method that utilizes compositions of nanoscale magnetic materials and target-specific ligands in conjunction with a device that provides alternating magnetic fields to treat diseased tissue by killing diseased cells via hyperthermia.

25      It is a further object of the present invention to provide methods for the treatment of diseased tissue, such as cancer, in a safe and effective manner, with minimal invasion, and short treatment periods.

The present invention pertains to magnetic material compositions that include magnetic particles forming a single magnetic domain; a biocompatible coating material for the particle; and a ligand that is selective to at least one cancer marker, and that can be bound to an uncoated portion of the particle, bound to a coated portion of the particle, bound to the particle and partially covered by the

coating, or intercalated into the coating. The present invention also pertains to devices for treating cancer by interacting with magnetic particles targeted to cancer cells in a patient. Such a device includes a magnetic generator having a core defining at least part of a magnetic circuit, two poles of the core defining a gap therebetween, a magnetic field passing between two poles, the gap being of sufficient size to receive a portion of the patient containing the cancer cells; and a power supply coupled to provide energy to the magnetic generator so that the magnetic field passing between the two poles alternates at a frequency of about 1 kHz or more.

The present invention further pertains to methods related to such magnetic material compositions and devices for the treatment of cancer. One such method includes the administration to the patient of a magnetic material composition that includes at least one, single domain, magnetic particle attached to a cancer-cell specific ligand, and application of an alternating magnetic field to a region of the patient containing the cancer so as to inductively heat the magnetic material composition and kill the cancer cells.

The therapeutic methods of the present invention provide for the treatment of diseased tissue, such as cancer, in a safe and effective manner, with minimal invasion, and short treatment periods.

The above summary of the present invention is not intended to describe each illustrated embodiment or every implementation of the present invention. The figures and the detailed description which follow more particularly exemplify these embodiments.

## 25 BRIEF DESCRIPTION OF THE DRAWINGS

The invention may be more completely understood in consideration of the following detailed description of various embodiments of the invention in connection with the accompanying drawings, in which:

Figure 1 schematically illustrates a thermotherapy treatment system according to an embodiment of the present invention;

**Figure 2** schematically illustrates a thermotherapy treatment according to an embodiment of the present invention;

**Figure 3** schematically illustrates a circuit for producing a thermotherapeutic alternating magnetic field according to an embodiment of the present invention;

5       **Figure 4a** graphically illustrates a thermotherapeutic sinusoidal current waveform according to an embodiment of the present invention;

**Figure 4b** graphically illustrates a thermotherapeutic triangular current waveform according to an embodiment of the present invention;

10      **Figure 5a** graphically illustrates a thermotherapeutic sinusoidal waveform modulation according to an embodiment of the present invention;

**Figure 5b** graphically illustrates a thermotherapeutic pulsed waveform modulation according to an embodiment of the present invention;

**Figure 6** schematically illustrates a thermotherapeutic bioprobe configuration according to an embodiment of the present invention;

15      **Figure 7** schematically illustrates a disease specific targeting ligand component of a bioprobe according to an embodiment of the present invention;

**Figure 8** schematically illustrates disease specific bioprobes bound to a disease cell surface according to an embodiment of the present invention;

20      **Figure 9a** schematically illustrates a thermotherapeutic treatment according to an embodiment of the present invention; and

**Figure 9b** schematically illustrates the design of a coil for use in the embodiment illustrated in **Figure 9a**.

25      While the invention is amenable to various modifications and alternative forms, specifics thereof have been shown by way of example in the drawings and will be described in detail. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

## DETAILED DESCRIPTION

The present invention pertains to magnetic material compositions, devices for treating diseased, disease-causing, or undesirable tissue or material, for use with magnetic material compositions, and methods for treating or removing the tissue or material utilizing such devices and magnetic material compositions. The therapeutic methods disclosed herein include the targeted delivery of nanometer sized magnetic particles to the desired or target material. The term "bioprobe", as used herein, refers to the composition including a magnetic particle, a biocompatible coating material, and a target-specific ligand. The term "ligand", as used herein, refers to compounds which target biological markers. Examples of ligands include proteins, peptides, antibodies, antibody fragments, saccharides, carbohydrates, glycans, cytokines, chemokines, nucleotides, lectins, lipids, receptors, steroids, neurotransmitters, Cluster Designation/Differentiation (CD) markers, imprinted polymers, and the like. The methods for treating diseased tissue disclosed herein include administering to a patient the bioprosbes suspended in an appropriate medium, and applying, via a device capable of interacting with the bioprosbes, an alternating magnetic field to an area of the patient containing the bioprosbes to heat the bioprosbes sufficiently to kill or render ineffective the disease material.

One embodiment of the invention, as illustrated in **Figure 1**, includes an alternating magnetic field (AMF) generator located within a cabinet **101** designed to produce an alternating magnetic field (AMF) that may be guided to a specific location within a patient **105** by a magnetic circuit **102**. The therapeutic methods of the present invention may be performed following a determination of the presence of disease material in one or more areas of the patient. For example, the disease material may be any one or combination of cancers and cancerous tissue, a pathogenic infection (viral, bacterial or multicellular parasitic), toxin, or any pathogen-like material (prion). The manner of making the diagnosis does not form part of the invention and may be performed using any standard method. However, the present invention, or aspects thereof, may be amenable to a diagnostic function alone or in conjunction with another method or apparatus. Such a diagnostic function would be performed by using a suitable technology or technique to

interrogate the magnetic properties of the bioprobes, and thus evaluate their concentration and location within the patient. Both the location and concentration of bioprobes may be determined using an existing technique such as magnetic resonance imaging, or another diagnostic technique can be established and

5 performed using a suitable magnetometer, such as a Superconducting Quantum Interference Device (SQUID). Information obtained from this interrogation may be used to define the parameters of treatment, i.e. the location, duration, and intensity, of the alternating magnetic field. The patient lies upon an X-Y horizontal and vertical axis positioning bed 106. The bed 106 is both horizontally and vertically

10 positionable via a bed controller 108. The AMF generator produces an AMF in the magnetic circuit 102 that exits the magnetic circuit at one pole face 104, passing through the air gap and the desired treatment area of the patient, and reenters the circuit through the opposing pole face 104, thus completing the circuit. An operator or medical technician is able to both control and monitor the AMF characteristics

15 and bed positioning via the control panel 120.

Figure 2 illustrates a treatment of a patient with a device for treating disease material according to an embodiment of the present invention. The area of the patient to be treated 205 is localized in the region between the magnetic poles 204 via the positionable bed 206. This region may be any location of the patient including the chest, abdomen, head, neck, back, legs, arms, any location of the skin. An AMF may be applied to the treatment area 205 of the patient, as illustrated by the magnetic lines of flux 212. The magnetic field, manifested by the magnetic lines of flux 212 interacts with both healthy and disease material in the localized area. Bioprobes 210, containing at least one appropriate ligand selective to the particular type of disease material, are bound to the disease material 214. In the illustrated case, the bioprobes 210 are selective to breast cancer. The bioprobes 210 become excited by the interacting applied AMF and are inductively heated to a temperature sufficient to kill or render ineffective the disease material. For example, heat generated in the bioprobes 210 may pass to the cells, thereby causing the cells to die.

20 Furthermore, the poles 204 may be formed from pieces whose gap is adjustable, so as to permit other parts of the body to be treated. It is advantageous to

set the gap between the poles 204 to be sufficiently large to permit the part of the body containing the disease material to enter the gap, but not be so large as to reduce the magnetic field strength. Also shown are secondary coils 208 and optional cores 209. Any number of these may be added to modify the distribution of magnetic flux produced by the primary coils and the core. The secondary coils 208 may be wired in series or in parallel with the primary coils, or they can be driven by separate AMF generators. The phase, pulse width and amplitude of the AMF generated by these coils may be adjusted to maximize the field strength in the gap, minimize the field strength in areas which may be sensitive to AMF, or to uniformly distribute the magnetic field strength in a desired manner.

Figure 3 illustrates a circuit for producing an AMF according to an embodiment of the present invention. The AMF generator 318 is supplied with alternating current (AC) power via the conduit 316. A circulating fluid supply is also provided in the conduit 316. The AMF generator 318 may become hot, and it may be cooled with the circulating fluid supply while in operation. The fluid may be water; however a fluid such as silicone oil or other inorganic or organic fluids with suitable thermal and electric properties may be preferable to increase generator efficiency. The energy produced by the generator 318 is directed through the AMF matching network 320 where the impedance of the generator is matched to the impedance of the coil 322. The impedance of the AMF matching network 320 may be adjustable to minimize the energy reflected back to the generator 318. In another embodiment, the generator frequency may be automatically adjusted to minimize the reflected energy. The modified energy may be directed to the magnetic circuit 302. An AMF is induced in the magnetic circuit 302 as a result of the current passing through the solenoid coil 322. Magnetic lines of flux 312 are produced in the gap 333 between the poles 304 in the magnetic circuit 302. Items 331 and 332 illustrate a liquid cooling send and return.

A feedback loop 324 may be provided for monitoring the magnetic field profile in the gap 333 between the poles 304. The probe 354 may provide data to the monitor 352, which relays information to the controller 356 via an appropriate data bus 324. Information from the controller 356 is relayed to the generator 318 via

an appropriate data bus 358. Monitoring the magnetic field profile may be useful in detecting the presence of magnetic particles, monitoring an inductance of tissue, and monitoring the temperature of tissue located in the gap 333.

Measuring alternating magnetic fields directly is extremely difficult.

5 Because the AMF is proportional to the current in the coil 322, characteristics of the AMF may be defined in terms of the coil current, which can readily be measured with available test equipment. For example, the coil current may be viewed and measured with a calibrated Rogowski coil and any oscilloscope of suitable bandwidth. The fundamental waveform may be observed as the direct measure of  
10 the magnitude and direction of the coil current. Many different types of fundamental waveforms may be used for the AMF. For example, **Figure 4a** illustrates a sinusoidal current waveform, and **Figure 4b** illustrates a triangular current waveform. The shape of the fundamental waveform may also be square, sawtooth, or trapezoidal.

15 Most practical generators produce an approximation of these waveforms with some amount of distortion. In most applications, this waveform may be nearly symmetrical around zero, as illustrated in **Figures 4a** and **4b**. However, there may be a static (DC) current, known as a DC offset, superimposed on the waveform. An AMF with a DC offset can be used to influence the movement of bioprobes within  
20 the body. With a suitable gradient and the “vibration-like” effect of the AC component, the bioprobes are typically drawn toward the area of highest field strength. **Figures 4a** and **4b** show at least one cycle of two different fundamental waveforms with zero or near zero DC offsets. The fundamental period may be defined as the time it takes to complete one cycle. The fundamental frequency may  
25 be defined as the reciprocal of the fundamental period. The fundamental frequency may be between 1 kHz and 1 GHz, preferably between 50 kHz and 15 MHz, and more preferably between 100 kHz and 500 kHz. The fundamental frequency may be intentionally modulated, and may often vary slightly as a result of imperfections in the RF generator design.

30 The amplitude of the waveform may also be modulated. **Figure 5a** illustrates an embodiment in which a sinusoidal current modulation envelope may be

used, and **Figure 5b** illustrates an embodiment that utilizes a square modulation envelope. The shape of the amplitude modulation envelope may typically be sinusoidal, square, triangular, trapezoidal or sawtooth, and may be any variation or combination thereof, or may be some other shape.

5       The AMF produced by the generator may also be pulsed. Pulse width is traditionally defined as the time between the -3dBc points of the output of a square law crystal detector. Because this measurement technique is cumbersome in this application, we use an alternate definition of pulse width. For the purpose of this invention, pulse width may be defined as the time interval between the 50%  
10      amplitude point of the pulse envelope leading edge and the 50% amplitude point of the pulse envelope trailing edge. The pulse width may also be modulated.

15     The pulse repetition frequency (PRF) is defined as the number of times per second that the amplitude modulation envelope is repeated. The PRF typically lies between 0.0017 Hz and 1000 MHz. The PRF may also be modulated. The duty cycle may be defined as the product of the pulse width and the PRF, and thus is dimensionless. In order to be defined as pulsed, the duty of the generator **318** must be less than unity (or 100%).

20     The AMF may be constrained to prevent heating healthy tissue to lethal temperatures, for example by setting the temperature of the tissue to be around 43 °C, thus allowing for a margin of error of about 3°C from the temperature of 46.5 °C that is lethal to healthy tissue. This may be accomplished in a variety of ways.

- The peak amplitude of the AMF may be adjusted.
- The PRF may be adjusted.
- The pulse width may be adjusted.
- The fundamental frequency may be adjusted.
- 25     • The treatment duration may be adjusted.

30     These four characteristics may be adjusted to maximize the heating rate of the bioprobes and, simultaneously, to minimize the heating rate of the healthy tissue located within the treatment volume. These conditions may vary depending upon tissue types to be treated, thus the operator may determine efficacious operation levels. In one embodiment, one or more of these characteristics may be adjusted

during treatment based upon one or more continuously monitored physical characteristics of tissue in the treatment volume by the probe 354, such as temperature or impedance. This information may then be supplied as input to the generator 318, via the monitor 352, the data bus 324, the controller 356, and the data bus 358 to control output, constituting the feedback loop. In another embodiment, one or more physical characteristics of the bioprobes (such as magnetic properties) may be monitored during treatment with a suitable device. In this case, one or more magnetic property, such as the magnetic moment, is directly related to the temperature of the magnetic material. Thus, by monitoring some combination of magnetic properties of the bioprobe, the bioprobe temperature can be monitored indirectly. This information may also be supplied as input to the generator 318, via the monitor 352, the data bus 324, the controller 356, and the data bus 358 to control output to become part of the feedback loop. The generator output may be adjusted so that the peak AMF strength is between about 10 and about 10,000 Oersteds (Oe).  
10 Preferably, the peak AMF strength is between about 20 and about 3000 Oe, and more preferably, between about 100 and about 2000 Oe.  
15

In another embodiment of the present invention, the differential heating of the bioprobes, as compared to that of the healthy tissue, may be maximized. The bioprobes 210 heat in response to each cycle of the AMF. Assuming the fundamental frequency, the PRF, and the pulse width remain constant, the heat output of the bioprobe 210 continues to increase as peak amplitude of the AMF increases until the magnetic material of the bioprobe reaches saturation. Beyond this point, additional increases in AMF amplitude yield almost no additional heating. At AMF amplitudes below saturation however, it can be said that bioprobe heating is a function of AMF amplitude. Unlike bioprobes, healthy tissue heating is a result of eddy current flow and a function of the rate of change of the AMF. In particular, the eddy current and resultant tissue heating following the expressions:  
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25

- (1)  $I_{\text{eddy}} \propto dB/dT$
- 30 (2) Tissue Heating  $\propto I_{\text{eddy}}^2$ .

From the relationships (1) and (2), it is evident that reducing the rate of change of the AMF yields a significant reduction in tissue heating. In one embodiment of the present invention, this relationship is exploited by using a symmetrical triangular wave, as shown in **Figure 4b**, as the fundamental waveform. By avoiding the high rates of change that occur as a sinusoid crosses the X-axis (**Figure 4a**), and substituting the constant but lower rate of change associated with a triangular waveform (**Figure 4b**), tissue heating may be reduced with little or no sacrifice in bioprobe heating. A triangular waveform, as shown in **Figure 4b**, may be achieved by using an appropriate generator, such as a linear amplifier-based generator. Some distortion of the triangle is inevitable, but tangible reductions in tissue heating result from even small reductions in dB/dT.

The heating of both the tissue and the bioprobes increase with increased AMF amplitude. At low AMF amplitudes, small increases yield significant increases in magnetic heating. As the bioprobes approach saturation however, their relationship with the AMF amplitude becomes one of diminishing return. This relationship is unique to the particular magnetic material, as are the values that constitute "low" or "saturating" AMF amplitudes. Bioprobe heating is at first related to the AMF amplitude by an exponent  $>1$ , which gradually diminishes to an exponent  $<1$  as saturation is approached. At typical pulse widths and duty cycles, eddy current heating is directly related to duty cycle. The capability to pulse the generator output, as illustrated in **Figures 5a or 5b**, allows the benefits of operating at higher AMF amplitudes while maintaining a constant reduced tissue heating by reducing the duty cycle.

It is desirable to apply the AMF to the treatment area **205** of the patient **105**. Generating high peak amplitude AMF over a large area requires a very large AMF generator and exposes large amounts of healthy tissue to unnecessary eddy current heating. Without some way of directing the field to where it is useful, disease in the chest or trunk may only be practically treated by placing the patient within a large solenoid coil. This would expose most of the major organs to eddy current heating, which must then be monitored and the AMF adjusted so as not to overheat any part

of a variety of tissue types. Each of these tissue types has a different rate of eddy current heating. The peak AMF strength would need to be reduced to protect those tissue types that experience the most extreme eddy current heating. If the varieties of exposed tissue are minimized, it is likely that the AMF strength can be increased,

5 and thereby reducing the treatment time and increasing the efficacy. One method of confining the high peak amplitude AMF to treatment area **205** is by defining the lowest reluctance path of magnetic flux with high permeability magnetic material. This path is referred to as a magnetic circuit (**102** in **Figure 1** and **302** in **Figure 3**). The magnetic circuit may be provided so that all or most of the magnetic flux

10 produced by the coil **322** may be directed to the treatment area **205**. One benefit of the magnetic circuit **302** is that the necessary amount of flux may be reduced since the amount of flux extending beyond the treatment area **205** is minimized. Reducing the required flux reduces the required size and power of the AMF generator, and minimizes exposure of tissue outside the treatment area **205** to high peak amplitude

15 AMF. In addition, a reduced area of AMF exposure avoids the unintentional heating of surgical or dental implants and reduces the likelihood that they will need to be removed prior to treatment, thereby avoiding invasive medical procedures.

Concentrating the field permits the treatment of large volumes within the chest or trunk with a portable size device.

20 The material used to fabricate the magnetic circuit **302** may be appropriate to the peak amplitude and frequency of the AMF. The material may be, but is not limited to, iron, powdered iron, assorted magnetic alloys in solid or laminated configurations and ferrites. The pole faces **104**, **204**, and **304** may be shaped and sized to further concentrate the flux produced in the treatment area. The pole faces

25 **304** may be detachable. Different pole pieces having different sizes and shapes may be used, so that the treatment area and volume may be adjusted. When passing from one material to another, the lines of magnetic flux **312** travel in a direction normal to the plane of the interface plane. Thus, the face **304** may be shaped to influence the flux path through gap **333**. The pole faces **304** may be detachable and may be

30 chosen to extend the magnetic circuit **302** as much as possible, to minimize gap the **333** while leaving sufficient space to receive that portion of the patient being treated.

As discussed above, the addition of secondary coils can aid in the concentration of the field as well as reducing the field strength in sensitive areas.

**Figure 6** discloses a bioprobe configuration according to an embodiment of the present invention. A spherical shaped bioprobe 690, having a magnetic particle 642 at its center, is shown. The magnetic particle 642 may be covered with a coating 644. At least one targeting ligand 640, such as, but not limited to, an antibody, may be located on an exterior portion of the bioprobe 690. The targeting ligand 640 may be selected to seek out and bond with a particular type of cell or disease matter. Heat is generated when the magnetic particle 642 of the bioprobe 690 is subjected to the AMF. In a general sense, this heat represents an energy loss as the magnetic properties of the material are forced to oscillate in response to the applied alternating magnetic field. The amount of heat generated per cycle of magnetic field and the mechanism responsible for the energy loss depend on the specific characteristics of both the magnetic material of the particle 642 and the magnetic field. The magnetic particle 642 heats to a unique temperature, known as the Curie temperature, when subjected to the AMF. The Curie temperature is the temperature of the reversible ferromagnetic to paramagnetic transition of the magnetic material. Below this temperature, the magnetic material heats in an applied AMF. However, above the Curie temperature, the magnetic material becomes paramagnetic and its magnetic domains become unresponsive to the AMF. Thus, the material does not generate heat when exposed to the AMF above the Curie temperature. As the material cools to a temperature below the Curie temperature, it recovers its magnetic properties and resumes heating, as long as the AMF remains present. This cycle may be repeated continuously during exposure to the AMF. Thus, magnetic materials are able to self-regulate the temperature of heating. The temperature to which the particle 642 heats is dependent upon, inter alia, the magnetic properties of the material, characteristics of the magnetic field, and the cooling capacity of the target site 214. Selection of the magnetic material and AMF characteristics may be tailored to optimize treatment efficacy of a particular tissue or target type. In an embodiment of the present invention, the magnetic material may be selected to possess a Curie temperature between 40 °C and 150 °C.

The magnetic attributes of ferromagnets, ferrites (ferrimagnets), and superparamagnets are determined by an ensemble of interacting magnetic moments in a crystalline structure. The magnetic moments of ferromagnets are parallel and equal in magnitude, giving the material a net magnetization, or net magnetization vector. By contrast, ferrites are ferrimagnetic, where adjacent magnetic moments are parallel in direction and unequal in magnitude, yielding a net magnetization in ferrimagnetic coupling. Superparamagnets possess clusters or collections of atomic magnetic moments that are either ferromagnetic or ferrimagnetic, however there may be no particular relationship in the orientation of the moments among several clusters. Thus, a superparamagnetic material may possess a net magnetic moment.

A magnetic domain may be defined as an area of locally saturated magnetization, and the magnetic domain boundary thickness, or the distance separating adjacent magnetic domains, may be about 100 nm. Thus, magnetic particles (ferromagnetic or ferrimagnetic) possessing a dimension smaller than 250 nm, and preferably less than about 100 nm, are single domain magnetic particles, where each particle is a magnetic dipole.

The mechanisms responsible for energy loss exhibited by single domain particles exposed to an alternating magnetic field are still not well understood, however a currently accepted description exists, which is included herein for clarity.

When a single domain particle is exposed to an AMF, the whole magnetic dipole rotates in response to the field with a concomitant energy loss liberated as heat. This mechanism is often referred to as the Ne  l mechanism. The external magnetic forces required for this intrinsic change in magnetization depend upon the anisotropy energy of the magnetic domain, size, and shape of the single domain particle.

Furthermore, it is currently accepted that there is a mechanical rotation of the entire single domain particle when exposed to an alternating magnetic field. This latter phenomenon, commonly called the Brownian mechanism, also contributes to the energy loss of a single domain particle, and is proportional to the viscosity of the material surrounding the particle. Thus, the coating 644 may enhance the heating properties of the bioprobe 690, particularly if the coating has a high viscosity, for example, if the coating is a polymer.

The heating mechanism responsible for the energy loss experienced by a single domain particle in an AMF can be clearly distinguished from the hysteresis heating of larger, or multidomain magnetic particles. Single domain particles of a given composition can produce substantially more heat per unit mass than multi-domain particles that are 1000 times larger (multi domain particles). The heating mechanism exhibited by single domain particles may be optimized to produce superior heating properties over larger particles for disease treatment. The amount of heat delivered to a cell may be tailored by controlling both the particle size and coating variation, as well as characteristics of the magnetic field, thereby providing a range of possible bioprobe compositions designed for tissue-specific treatments.

Many aspects of the magnetic particle **642**, such as material composition, size, and shape, directly affect heating properties. Many of these characteristics may be designed simultaneously to tailor the heating properties for a particular set of conditions found within a tissue type. For example, first considering the magnetic particle **642**, the most desirable size range depends upon the particular application and on the material(s) comprising the magnetic particle **642**.

The size of the magnetic particle **642** determines the total size of the bioprobe **690**. Bioprosbes **690** that are to be injected may be spherical and may be required to have a long residence time in the bloodstream, i.e., avoid sequestration by the liver and other non-targeted organs. The bioprobe **690** may be successful in avoiding sequestration if its total diameter is less than about 30 nm. If the bioprobe **690** contains a magnetite ( $Fe_3O_4$ ) particle **642**, then a preferred diameter of the magnetic particle **642** may be between about 8 nm and about 20 nm. In this case, the bioprosbes **690** may be sufficiently small to evade the liver, and yet the magnetic particle **642** still retains a sufficient magnetic moment for heating. Magnetite particles larger than about 8 nm generally tend to be ferrimagnetic and thus appropriate for disease treatment. If other elements, such as cobalt, are added to the magnetite, this size range can be smaller. This results directly from the fact that cobalt generally possesses a larger magnetic moment than magnetite, which contributes to the overall magnetic moment of the cobalt-containing magnetic

particle 642. In general, the preferred size of the bioprobe 690 may be about 0.1 nm to about 250 nm, depending upon the disease indication and bioprobe composition.

While determining the size of the magnetic particle 642, its material composition may be determined based on the particular target. Because the self-limiting temperature of a magnetic material, or the Curie temperature, is directly related to the material composition, as is the total heat delivered, magnetic particle compositions may be tuned to different tissue or target types. This may be required because each target type, given its composition and location within the body, possesses unique heating and cooling capacities. For example, a tumor located within a region that is poorly supplied by blood and located within a relatively insulating region may require a lower Curie temperature material than a tumor that is located near a major blood vessel. Targets that are in the bloodstream will require different Curie temperature materials as well. Thus, in addition to magnetite, particle compositions may contain elements such as cobalt, iron, rare earth metals, etc.

The presence of the coating 644 and the composition of the coating material may form an integral part of the energy loss, and thus the heat produced, by the bioprosbes 690. In addition, the coating 644 surrounding the particles may serve additional purposes. Its most important role may be to provide a biocompatible layer separating the magnetic material from the immunologic defenses in a patient, thereby controlling the residence time of the particles in the blood or tissue fluids.

For example, this control of residence time allows one to choose targeting ligands 640 that are best suited for a particular tissue type. In addition, the coating 644 may serve to protect the patient from potentially toxic elements in the magnetic particle 642. A second function of the coating materials may be the prevention of particle aggregation, as the bioprosbes 690 may be suspended in a fluid. It may be also be advantageous to coat the bioprobe 690 with a biocompatible coating that is biodegradable. In such an application, both the coating 644 and the magnetic particle 642 may be digested and absorbed by the body.

Suitable materials for the coating 644 include synthetic and biological polymers, copolymers and polymer blends, and inorganic materials. Polymer

materials may include various combinations of polymers of acrylates, siloxanes, styrenes, acetates, alkylene glycols, alkylenes, alkylene oxides, parylenes, lactic acid, and glycolic acid. Further suitable coating materials include a hydrogel polymer, a histidine-containing polymer, and a combination of a hydrogel polymer and a histidine-containing polymer.

Coating materials may include combinations of biological materials such as a polysaccharide, a polyaminoacid, a protein, a lipid, a glycerol, and a fatty acid. Other biological materials for use as a coating material may be a heparin, heparin sulfate, chondroitin sulfate, chitin, chitosan, cellulose, dextran, alginate, starch, carbohydrate, and glycosaminoglycan. Proteins may include an extracellular matrix protein, proteoglycan, glycoprotein, albumin, peptide, and gelatin. These materials may also be used in combination with any suitable synthetic polymer material.

Inorganic coating materials may include any combination of a metal, a metal alloy, and a ceramic. Examples of ceramic materials may include a hydroxyapatite, silicon carbide, carboxylate, sulfonate, phosphate, ferrite, phosphonate, and oxides of Group IV elements of the Periodic Table of Elements. These materials may form a composite coating that also contains any biological or synthetic polymer. Where the magnetic particle **642** is formed from a magnetic material that is biocompatible, the surface of the particle itself operates as the biocompatible coating.

The coating material may also serve to facilitate transport of the bioprobe **690** into a cell, a process known as transfection. Such coating materials, known as transfection agents, include vectors, prions, polyaminoacids, cationic liposomes, amphiphiles, and non-liposomal lipids or any combination thereof. A suitable vector may be a plasmid, a virus, a phage, a viro, a viral coat. The bioprobe coating may be a composite of any combination of transfection agent with organic and inorganic materials, such that the particular combination may be tailored for a particular type of a diseased cell and a specific location within a patient's body.

To ensure that the bioprobe **690** selectively attaches to the target, an appropriate ligand **640** may be combined with the bioprobe **690**. The association of a ligand or ligands with the bioprobe **690** allows for targeting of cancer or disease markers on cells. It also allows for targeting biological matter in the patient. The

term ligand relates to compounds which may target molecules including, for example, proteins, peptides, antibodies, antibody fragments, saccharides, carbohydrates, glycans, cytokines, chemokines, nucleotides, lectins, lipids, receptors, steroids, neurotransmitters, Cluster Designation/Differentiation (CD) markers, and imprinted polymers and the like. The preferred protein ligands include, for example, cell surface proteins, membrane proteins, proteoglycans, glycoproteins, peptides and the like. The preferred nucleotide ligands include, for example, complete nucleotides, complimentary nucleotides, and nucleotide fragments. The preferred lipid ligands include, for example phospholipids, glycolipids, and the like. The ligand **640** may be covalently bonded to or physically interacted with the magnetic particle **642** or the coating **644**. The ligand **640** may be bound covalently or by physical interaction directly to an uncoated portion of the magnetic particle **642**. The ligand **640** may be bound covalently or by physical interaction directly to an uncoated portion of the magnetic particle **642** and partially covered by the coating **644**. The ligand **640** may be bound covalently or by physical interaction to a coated portion of the bioprobe **690**. The ligand **640** may be intercalated to the coated portion of the bioprobe **690**.

Covalent bonding may be achieved with a linker molecule. The term "linker molecule," as used herein, refers to an agent that targets particular functional groups on the ligand **640** and on the magnetic particle **642** or the coating **644**, and thus forms a covalent link between any two of these. Examples of functional groups used in linking reactions include amines, sulfhydryls, carbohydrates, carboxyls, hydroxyls and the like. The linking agent may be a homobifunctional or heterobifunctional crosslinking reagent, for example, carbodiimides, sulfo-NHS esters linkers and the like. The linking agent may also be an aldehyde crosslinking reagent such as glutaraldehyde. The linking agent may be chosen to link the ligand **640** to the magnetic particle **642** or the coating **644** in a preferable orientation, specifically with the active region of the ligand **640** available for targeting. Physical interaction does not require the linking molecule and the ligand **640** be bound directly to the magnetic particle **642** or to the coating **644** by non-covalent means such as, for example, absorption, adsorption, or intercalation.

Figure 7 schematically shows an example of a ligand that may be used with an embodiment of the present invention. The ligand may be an antibody having a fragment crystallization (Fc) region 760 and fragment antigen binding (Fab) regions 772. The Fab regions 772 may be the antigen binding regions of the antibody that include a variable light region 764 and a constant light region 766 along with a variable heavy region 768 and a constant heavy region 770. Biological activity of antibodies may be determined to a large extent by the Fc region 760 of the antibody molecule. The Fc region may include complement activation constant heavy chains 782 and macrophage binding constant heavy chains 784. The Fc region 760 and Fab regions 772 may be connected by several disulfide linkages 762. Ligands that do not include the Fc region 760 may be preferable in order to avoid immunogenic response. Examples of these ligands may include antibody fragments such as, fragment antigen binding fragments (fabs) 772, disulfide-stabilized variable region fragments (dsFVs) 774, single chain variable region fragments (scFVs) 780, recombinant single chain antibody fragments, and peptides.

An antigen binding fragment (Fab) 772 may include a single Fab region 772 of an antibody. A single Fab region may include a variable light 764 and a constant light region 766 bound to a variable heavy 768 and a constant heavy region 770 by a disulfide bond 762.

A disulfide-stabilized variable region fragment (dsFV) 774 may include a variable heavy region 768 and a variable light region 764 of antibody joined by a disulfide bond. A leader sequence 776, which may be a peptide, may be linked to the variable light 764 and variable heavy regions 768.

A single chain variable region fragment (scFV) 780 may include a variable heavy region 768 and variable light region 764 of antibody joined by a linker peptide 778. A leader sequence 776 may be linked to the variable heavy region 768.

A preferred ligand embodiment may include, for example, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, recombinant antibodies, bispecific antibodies, antibody fragments, scFVs 780, Fabs 772, dsFVs 774, recombinant single chain antibody fragments, peptides, and the like. Bispecific antibodies are non-natural antibodies

that bind two different epitopes that are typically chosen on two different antigens. A bispecific antibody is typically comprised of two different fragment antigen binding regions (Fabs) 772. A bispecific antibody may be formed by cleaving an antibody into two halves by cleaving the disulfide bonds 762 in the Fc region 782 only. Two antibody halves with different Fab regions 772 are then combined to form a bispecific antibody with the typical "Y" structure. One or more ligands can be present in the bioprobe formulation. Antibodies of virtually any origin may be used according to this embodiment, provided they bind the target, although human, chimeric, and humanized antibodies may aid in avoiding the patient's immunogenic response.

In another embodiment, the ligand 640 may be designed to target a specific cancer cell marker or markers. The particular cancer cell marker and ligand(s) 640 may be specific to, but not limited to, the type and location of the cancer such as, for example, tumors, metastatic cancer, minimal residual disease and the like. The ligand(s) 640 may have an affinity for the cancer marker or markers of interest. The cancer marker or markers may be selected such that they represent a viable target on the cancer cells of interest. The preferred cancer marker may be expressed on the surface of the cancer cells and is preferably present in very low amounts or not at all in normal cells. The preferred cancer marker may not be readily shed from the surface, or if shed, the ligand on the bioprobe may recognize a particular epitope of the marker that remains on the cell surface.

Figure 8 discloses an embodiment of the present invention wherein the bioproses 890 include a magnetic particle 842 and a coating material 844 and are bound to the cell 0000surface (wall) 846 by the ligand 840. The cell wall 846 may express several types of chemical species 848 and 850 that may serve as markers. The specificity of the bioproses 890 may be represented by attachment to the targeted cell marker 850 over the many other markers or molecules 848 on the cell surface 846. One or more bioproses may be attached to the cell surface 846 via the ligand 840. The ligands 840 may be adapted and the bioproses 890 may be designed such that the bioproses 890 remain on the cell surface 846 or they may be internalized into the cell. Once bound to the cell surface 846, the magnetic particle

842 of the bioprobe 890 heats in response to the AMF. The heat may emanate through the coating material 844 or through interstitial regions via convection, conduction, radiation, or any combination of these heat transfer mechanisms to the cell surface 846. The heated cell surface 846 becomes damaged, preferably in a 5 manner that causes irreparable damage to the cell. When a bioprobe(s) 890 becomes internalized within the cell, the bioprobe heats the cell internally via convection, conduction, radiation, or any combination of these heat transfer mechanisms. When a sufficient amount of heat is transferred by the bioprosbes 890 to the cell, the cell dies by necrosis, apoptosis or another mechanism.

10       The choice of a marker (antigen) 850 may be important to therapy utilizing bioprosbes. For breast cancer and its metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, members of the MUC-type mucin family, an epithelial growth factor (EGFR) receptor, a carcinoembryonic antigen (CEA), a human carcinoma antigen, a vascular endothelial growth factor (VEGF) antigen, a melanoma antigen (MAGE) gene, family antigen, a T/Tn antigen, a hormone receptor, growth factor receptors, a cluster designation/differentiation (CD) antigen, a tumor suppressor gene, a cell cycle regulator, an oncogene, an oncogene receptor, a proliferation marker, an adhesion molecule, a proteinase involved in degradation of extracellular matrix, a malignant transformation related 15 factor, an apoptosis related factor, a human carcinoma antigen, glycoprotein antigens, DF3, 4F2, MGFM antigens, breast tumor antigen CA 15-3, calponin, cathepsin, CD 31 antigen, proliferating cell nuclear antigen 10 (PC 10), and pS2. 20

25       For other forms of cancer and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a member of vascular endothelial growth factor receptor (VEGFR) family, a member of carcinoembryonic antigen (CEA) family, a type of anti-idiotypic mAB, a type of ganglioside mimic, a member of cluster designation/differentiation antigens, a member of epidermal growth factor receptor (EGFR) family, a type of a cellular adhesion molecule, a member of MUC-type mucin family, a type of cancer antigen 30 (CA), a type of a matrix metalloproteinase, a type of glycoprotein antigen, a type of melanoma associated antigen (MAA), a proteolytic enzyme, a calmodulin, a member

of tumor necrosis factor (TNF) receptor family, a type of angiogenesis marker, a melanoma antigen recognized by T cells (MART) antigen, a member of melanoma antigen encoding gene (MAGE) family, a prostate membrane specific antigen (PMSA), a small cell lung carcinoma antigen (SCLCA), a T/Tn antigen, a hormone receptor, a tumor suppressor gene antigen, a cell cycle regulator antigen, an oncogene antigen, an oncogene receptor antigen, a proliferation marker, a proteinase involved in degradation of extracellular matrix, a malignant transformation related factor, an apoptosis-related factor, a type of human carcinoma antigen.

In an embodiment of the invention, a bioprobe may include ligand(s) targeting the MUC-1 receptor of the mucin family. In yet another embodiment, a bioprobe has ligand(s) targeting at least one of the EGFR family such as Her-1, Her-2, Her-3 and Her-4. MUC-1 (Human epithelial mucin, CD277), is a high molecular weight, transmembrane mucin glycoprotein expressed by most glandular and epithelial cell lineages. In addition, MUC-1 has a large extracellular domain, portions of which may be shed into the bloodstream. MUC-1 may have a protective role, as its extracellular domain forms elongated rigid structures extending above other molecules on the cell. MUC-1 also plays a role in cell-cell and cell-substrate adhesion. MUC-1 is highly expressed in many human adenocarcinomas, including 80% of breast cancers, and is associated with poor prognosis. Mucin (MUC-1 and MUC-2) expression is associated with tumor invasiveness. MUC-1 and MUC-2 expression is associated with invasive ductive carcinoma of the breast. MUC-1 is also present at high levels on many myelomas. Different tissues/cells produce differing glycoforms of MUC-1. Glycosylation of MUC-1 in malignant cells is often altered compared to normal tissue. MUC-1 is considered a truly tumor specific antigen, although it is also found on normal cells, its aberrant glycosylation on tumors creates new epitopes for targeting. The extracellular domain of MUC-1 may be shed into the blood stream. The ligand may target the unshed remainder of the MUC-1 expressed on the cell surface.

Overexpression of growth factor receptors such as the EGFR family is indicated in tumors and has been associated with increased cell resistance to the cytotoxic effects of macrophages and cytotoxic factors, such as TNF (tumor necrosis

factor), which can lead to tumor growth. The protein encoded by the Her-1/neu gene is a 170,000 Dalton protein, referred to as Her-1. The protein encoded by the Her-2/neu gene is a 185,000 Dalton protein referred to as Her-2. Both proteins have an intracellular domain, a transmembrane domain that includes two cysteine-rich repeat clusters, and an intracellular kinase domain. The extracellular domain of Her-2 may be shed into the bloodstream. Thus, the ligand may target the unshed remainder of the Her-2 expressed on the surface of the cell.

For ovarian cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, one of ERBB2 (Her-2) antigen and CD64 antigen. For ovarian and/or gastric cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a polymorphic epithelial mucin (PEM). For ovarian cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, one of cancer antigen 125 (CA125) or matrix metalloproteinase 2 (MMP-2). For gastric cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, one of CA19-9 antigen and CA242 antigen.

For non-small-cell lung cancer (NSCLC), colorectal cancer (CRC) and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, vascular endothelial growth factor receptor (VEGFR), anti-idiotypic mAb, and carcinoembryonic antigen (CEA) mimic. For at least one of small-cell lung cancer (SCLC), malignant melanoma, and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, anti-idiotypic mAB or GD3 ganglioside mimic. For melanoma cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a melanoma associated antigen (MAA). For small cell lung cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a small cell lung carcinoma antigen (SCLCA).

For colorectal cancer (CRC) and/or locally advanced or metastatic head and/or neck cancer, a specific marker or markers may be chosen from cell surface markers such as, for example, epidermal growth factor receptor (EGFR). For Duke's

colorectal cancer (CRC) and its metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, Ep-CAM antigen.

For non-Hodgkin's lymphoma (NHL) and its metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, cluster designation/ differentiation(CD) 20 antigen or CD22 antigen. For B-cell chronic lymphocytic leukaemia and associated metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, CD52 antigen. For acute myelogenous leukaemia and its metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, CD33 antigen.

10 For prostate cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, prostate membrane specific antigen (PMSA). For carcinomatous meningitis and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, one of a vascular endothelial growth factor receptor (VEGFR) or an epithelial associated glycoprotein, for example, HMFGI (human milk fat globulin) antigen.

15 For lung, ovarian, colon, and melanoma cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, B7-H1 protein. For colon, breast, lung, stomach, cervix, and uterine cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, TRAIL Receptor-1 protein, a member of the tumor necrosis factor receptor family of proteins. For ovarian, pancreatic, non-small cell lung, breast, and head and neck cancers and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, EGFR (epidermal growth factor receptor).

20 For anti-angiogenesis targeting of tumor blood supply, a specific marker or markers may be chosen from cell surface markers such as, for example, Integrin  $\alpha v \beta 3$ , a cell surface marker specific to endothelial cells of growing blood vessels.

25 For targeting of colon and bladder cancer and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example,

RAS, a signaling molecule that transmits signals from the external environment to the nucleus. A mutated form of RAS is found in many cancers.

In another embodiment, the ligand **640** may be targeted to a predetermined target associated with a disease of the patient's immune system. The particular target and ligand(s) **640** may be specific to, but not limited to, the type of the immune disease. The ligand(s) **640** may have an affinity for a cell marker or markers of interest. The marker or markers may be selected such that they represent a viable target on T cells or B cells of the patient's immune system. The ligand(s) **640** may have an affinity for a target associated with a disease of the patient's immune system such as, for example, a protein, a cytokine, a chemokine, an infectious organism, and the like.

For diseases of the patient's immune system, a specific marker or markers may be chosen from cell surface markers. The targeted cells may be T or B cells of the immune system. For rheumatoid arthritis, a specific marker or markers may be chosen from cell surface markers such as, for example, one of CD52 antigen, tumor necrosis factor (TNF), and CD25 antigen. For rheumatoid arthritis and/or vasculitis, a specific marker or markers may be chosen from cell surface markers such as, for example, CD4 antigen. For vasculitis, a specific marker or markers may be chosen from cell surface markers such as, for example, CD18 antigen. For multiple sclerosis, a specific marker or markers may be chosen from cell surface markers such as, for example, CD52 antigen.

In another embodiment, the ligand **640** may be targeted to a predetermined target associated with a pathogen-borne condition. The particular target and ligand(s) **640** may be specific to, but not limited to, the type of the pathogen-borne condition. A pathogen is defined as any disease-producing agent such as, for example, a bacterium, a virus, a microorganism, a fungus, and a parasite. The ligand(s) **640** may have an affinity for the pathogen or pathogen associated matter. The ligand(s) **640** may have an affinity for a cell marker or markers associated with a pathogen-borne condition. The marker or markers may be selected such that they represent a viable target on infected cells.

For a pathogen-borne condition, the ligand(s) **640** for therapy utilizing bioprobes may be chosen to target the pathogen itself. For a bacterial condition, a predetermined target may be the bacteria itself, for example, one of *Escherichia coli* or *Bacillus anthracis*. For a viral condition, a predetermined target may be the virus itself, for example, one of Cytomegalovirus (CMV), Epstein-Barr virus (EBV), a hepatitis virus, such as Hepatitis B virus, human immunodeficiency virus, such as HIV, HIV-1, or HIV-2, or a herpes virus, such as Herpes virus 6. For a parasitic condition, a predetermined target may be the parasite itself, for example, one of *Trypanasoma cruzi*, Kinetoplastid, *Schistosoma mansoni*, *Schistosoma japonicum* or *Schistosoma brucei*. For a fungal condition, a predetermined target may be the fungus itself, for example, one of *Aspergillus*, *Cryptococcus neoformans* or *Rhizomucor*.

For a pathogen-borne condition, the ligand(s) **640** for therapy utilizing bioprobes may be chosen to target cell markers of pathogen infected cells. For the HIV virus, the predetermined target may be CTLA4 expressed on the surface of HIV infected T cells. CTLA4 migrates to the infected cell's outer surface when the HIV virus is ready to be released.

In another embodiment, the ligand **640** may be targeted to a predetermined target associated with an undesirable target. The particular target and ligand(s) **640** may be specific to, but not limited to, the type of the undesirable target. An undesirable target is a target that may be associated with a disease or an undesirable condition, but also present in the normal condition. For example, the target may be present at elevated concentrations or otherwise be altered in the disease or undesirable state. The ligand(s) **640** may have an affinity for the undesirable target or for biological molecular pathways related to the undesirable target. The ligand(s) **640** may have an affinity for a cell marker or markers associated with the undesirable target.

For an undesirable target, the choice of a predetermined target may be important to therapy utilizing bioprobes. The ligand(s) **640** may be chosen to target biological matter associated with a disease or undesirable condition. For arteriosclerosis, a predetermined target may be, for example, apolipoprotein B on

low density lipoprotein (LDL). For obesity, a predetermined marker or markers may be chosen from cell surface markers such as, for example, one of gastric inhibitory polypeptide receptor and CD36 antigen. Another undesirable predetermined target may be clotted blood.

5        In another embodiment, the ligand **640** may be targeted to a predetermined target associated with a reaction to an organ transplanted into the patient. The particular target and ligand(s) **640** may be specific to, but not limited to, the type of organ transplant. The ligand(s) **640** may have an affinity for a biological molecule associated with a reaction to an organ transplant. The ligand(s) **640** may have an 10      affinity for a cell marker or markers associated with a reaction to an organ transplant. The marker or markers may be selected such that they represent a viable target on T cells or B cells of the patient's immune system.

15      For reaction to a transplanted organ, the ligand(s) **640** may be chosen to target the immune response to a transplant. The transplanted organ may be treated before or after transplantation. For kidney transplantation, a predetermined marker or markers may be chosen from cell surface markers such as, for example, human T cell receptor (CD3) antigen or CD18 antigen. For kidney and bone marrow transplantation, a predetermined marker or markers may be chosen from cell surface markers such as, for example, CD52 antigen. For liver and bone marrow 20      transplantation, a predetermined marker or markers may be chosen from cell surface markers such as, for example, one of CD154 antigen and CD8 antigen. For transplantation tolerance, a predetermined marker or markers may be chosen from cell surface markers such as, for example, CD4. For bone marrow, a predetermined marker or markers may be chosen from cell surface markers such as, for example, 25      CD52 antigen for efficient depletion of T cells from bone marrow before transplantation in order to avoid graft versus host disease. For xenotransplantation or xenografting, a predetermined marker or markers may be, for example, galactose. Galactose is known to be on pig organs, but is not present in humans.

30      In another embodiment, the ligand **640** may be targeted to a predetermined target associated with a toxin in the patient. A toxin is defined as any poison produced by an organism including, but not limited to, bacterial toxins, plant toxins,

insect toxin, animal toxins, and man-made toxins. The particular target and ligand(s) **640** may be specific to, but not limited to, the type of toxin. The ligand(s) **640** may have an affinity for the toxin or a biological molecule associated with a reaction to the toxin. The ligand(s) **640** may have an affinity for a cell marker or markers associated with a reaction to the toxin.

For a toxin in the patient, the ligand(s) **640** may be chosen to target the toxin. A bacterial toxin target may be, for example, one of Cholera toxin, Diphtheria toxin, and Clostridium botulinus toxin. An insect toxin may be, for example, bee venom. An animal toxin may be, for example, snake toxin, for example, *Crotalus durissus terrificus* venom.

In another embodiment, the ligand **640** may be targeted to a predetermined target associated with a hormone-related disease. The particular target and ligand(s) **640** may be specific to, but not limited to, a particular hormone disease. The ligand(s) **640** may have an affinity for a hormone or a biological molecule associated with the hormone pathway. The ligand(s) **640** may have an affinity for a cell marker or markers associated with the hormone disease.

For a hormone related disease, the ligand(s) **640** may be chosen to a cell marker or markers. For estrogen-related disease or conditions, a predetermined target may be, for example, estrogen or cell surface marker or markers such as, for example, estrogen receptor. For human growth hormone disease, the predetermined target may be, for example, human growth hormone.

In another embodiment, the ligand **640** may be targeted to a predetermined target associated with non-cancerous diseased tissue. The particular target and ligand(s) **640** may be specific to, but not limited to, a particular non-cancerous diseased tissue. The ligand(s) **640** may have an affinity for a biological molecule associated with the non-cancerous diseased tissue. The ligand(s) **640** may have an affinity for a cell marker or markers associated with the non-cancerous diseased tissue.

For non-cancerous diseased tissue, the ligand(s) **640** may be chosen to be a predetermined target such as, for example, one of non-cancerous diseased deposits

and precursor deposits. For Alzheimer's disease, a predetermined target may be, for example, amyloid B protein and its deposits or apolipoprotein and its deposits.

In another embodiment, the ligand **640** may be targeted to a proteinaceous pathogen. The particular target and ligand(s) **640** may be specific to, but not limited to, a particular proteinaceous pathogen. The ligand(s) **640** may have an affinity for a proteinaceous pathogen or a biological molecule associated with the proteinaceous pathogen. The ligand(s) **640** may have an affinity for a cell marker or markers associated with the proteinaceous pathogen. For prion diseases also known as transmissible spongiform encephalopathies, a predetermined target may be, for example, Prion protein 3F4.

At least two approaches may be pursued for exposure of diseased material to the bioprobe therapy: treatment within the patient (intracorporeal) and treatment external to the patient (extracorporeal). In extracorporeal hyperthermal therapy, bioprobes may be used to lyse, denature, or otherwise destroy the desired target cells or antigens by circulating the blood outside of the body, exposing it to AMF, and returning it to the body. The bioprobes may be introduced into the vascular circulating system or into the blood circulating outside of the body.

For example, to target bioprobe/target complexes that are carried primarily in the blood serum or plasma, the blood serum or plasma may be extracorporeally separated from the other blood components, exposed to AMF to destroy the target, and recombined with the other blood components before returning the blood to the body. The bioprobes may be introduced into the vascular circulating system, the blood circulating outside of the body, or the blood serum or plasma after it is separated. In another embodiment, the bioprobes may be contained in a vessel or column through which the blood circulating outside of the body or the blood serum or plasma flows. The vessel or column may be exposed to AMF to destroy the targeted cells or antigens before returning the blood to the body.

All of the indications described above may be targeted using either treatment within the patient (intracorporeal) and treatment external to the patient (extracorporeal).

A method of administering the bioprobes **890** to the desired area for treatment and the dosage may depend upon, but is not limited to, the type and location of the diseased material. The size range of the bioprobes **890** allows for microfiltration for sterilization. An administration method may be, for example,

5 wash, lavage, as a rinse with sponge, or other surgical cloth as a perisurgical administration technique. Other methods of administration may include intravascular injection, intravenous injection, intraperitoneal injection, subcutaneous injection, and intramuscular injection. The bioprobes **890** may be formulated in an injectable format (suspension, emulsion) in a medium such as, for example, water, saline, Ringer's solution, dextrose, albumin solution, and oils. The bioprobes may

10 also be administered to the patient through topical application via a salve or lotion, transdermally through a patch, orally ingested as a pill or capsule or suspended in a liquid or rectally inserted in suppository form. Bioprobes may also be suspended in an aerosol or pre-aerosol formulation suitable for inhalation via the mouth or nose.

15 Once administered to the patient, delivery of the bioprobes **890** to the target site may be assisted by an applied static magnetic field due to the magnetic nature of the bioprobes. Assisted delivery may depend on the location of the targeted cancer. The bioprobes may also be delivered to the patient using other methods. For example, the bioprobes may be administered to the patient orally, or may be

20 administered rectally.

Another method of administering the bioprobes **890** to the desired area for treatment may include administering the magnetic material **642** composition and the ligand **640** to the patient separately and combining the ligand with the magnetic material in the patient's body. The ligand may be chosen to recognize a predetermined target. A ligand having a binder may be administered to the patient separately from the administration of a magnetic particle having a receptor to the binder. The ligand with the binding molecule may combine with the magnetic particle with receptor through the binder/receptor interaction in the patient's body to form a targeted bioprobe for therapy. In another embodiment, a ligand having a receptor may be administered to the patient separately from the administration of a magnetic particle having a binder. The ligand with the receptor may combine with

the magnetic particle with the binding molecule through the binder/receptor interaction in the patient's body to form a targeted bioprobe for therapy.

In one approach, a ligand having a binder to a target molecule may be administered to the patient separately from the administration of a magnetic particle conjugated to a target molecule. The ligand having the binder may combine with the magnetic particle with the target molecule through an interaction between the binder and the target molecule in the patient's body to form a targeted bioprobe for therapy. The administration of the ligand separate from the magnetic particle may allow for higher doses of ligand or multiple doses of the ligand to be given to the patient and may allow for time for the ligand to localize at the predetermined target. The magnetic particle may then be added to combine with the ligand at an appropriate time before bioprobe therapy.

In another approach, a biotinylated ligand and a streptavidin conjugated magnetic particle may be administered to the body separately and at separate times. The biotinylated ligand and the streptavidin conjugated magnetic particle may combine in the patient's body prior to bioprobe therapy. Also, for example, a streptavidin conjugated ligand and a biotinylated magnetic particle may be administered to the body separately and at separate times. The streptavidin conjugated ligand and the biotinylated magnetic particle may combine in the patient's body prior to bioprobe therapy. For example, the ligand may be a fusion protein comprised of a target-binding fragment combined with streptavidin and the magnetic particle may be biotinylated. The ligand may be administered separately to the patient and the target-binding fragment may be designed for a predetermined target for bioprobe therapy. The biotinylated magnetic particle may be administered separately and combine with the streptavidin fusion protein in the patient's body.

Another method of administering the bioproses **890** to the desired area for treatment may include administering the magnetic material **642** composition and the ligand **640** to the patient separately and combining the ligand with the magnetic material in the patient's body. A bispecific ligand may be administered to the patient separately from the administration of a magnetic particle conjugated to a target molecule. The bispecific ligand may be designed to recognize a

- predetermined target for therapy and also to recognize a target molecule. The bispecific ligand may combine with the magnetic particle conjugated to a target molecule in the patient's body to form a targeted bioprobe for therapy. For example, a bispecific antibody may be used to recognize the predetermined target and to also 5 recognize streptavidin. The bispecific antibody and a streptavidin conjugated magnetic particle may be administered to the patient separately and at a separate time or times. The bispecific ligand may combine with the magnetic particle conjugated to streptavidin through the affinity interactions in the patient's body to form a targeted bioprobe for therapy.
- 10 Once administered by any of the above methods, the movement of the bioprosbes can be influenced by an AMF with a DC offset and a suitable gradient, or even by a simple DC magnetic field with a suitable gradient. The magnetic field pattern may also be influenced to separate the patient from an electric field component associated with the magnetic field to reduce an undesirable dielectric 15 heating of patient tissue or cells.

**Figures 9a and 9b** disclose a treatment device for carrying out the thermotherapeutic treatment according to another embodiment of the present invention. The treatment device illustrated in **Figures 9a and 9b** includes at least one pancake coil (**909a** and **909b**) and a core (**905a** and **905b**). The treatment 20 device may also include a liquid cooling input (**901a** and **901b**) output (**902a** and **902b**), as well as a cooling circuit **903a**. An AC current applied to the coil (**909a** and **909b**), as described above, yields an AMF with a field shape approximated by the field lines **907a**. The treatment device is applicable for treating areas close to the surface, for example, the skin or may be applied with sufficient magnetic field 25 strength to treat the patient's muscle and bone tissue. The coil (**909a** and **909b**) may be coated with an appropriate insulating material for placement directly on the skin of a patient. In **Figure 9a**, the magnetic field generating device **900a** is shown treating undesirable tissue along the patient's leg **910a**. For treatment areas associated with target tissues such as melanoma or cellulite, the apparatus applies 30 sufficient field strength to kill undesirable tissue while limiting exposure to a non-targeted tissue.

Figures 9b schematically illustrates a face of a magnetic generating device 900b. A cross sectional view of the coil illustrated in Figure 9a is disclosed along axis A-A in Figure 9b. The device 900b is shown provided with a core 905b and a coil 909b from which, in operation, the magnetic field lines emanate. When 5 assembled, most of the magnetic field lines 907a, as illustrated in Figure 9a, appear to be touching the leg 910a. The magnetic field will be most intense close to the coil 909a and will diminish exponentially as the distance from the coil 909a increases. This characteristic provides for high field strength in the tissue near the surface while minimizing the exposure of deeper tissues.

10

### Examples

Having generally described the invention, a more complete understanding thereof may be obtained by reference to the following examples that are provided for purposes of illustration only and do not limit the invention.

15

#### Example 1: Identify Ferromagnetic Particles with Proper Curie Temperatures.

For magnetic materials, the saturation magnetization ( $4\pi M_s$ ) and the Curie temperatures ( $T_c$ ) depend on the material composition. The Curie temperature of the material may lie in the range 40 °C - 150 °C, although it may also lie outside this 20 range. For example, manganese alloys of the general formula  $RMn_2X$ , where R is a rare earth, such as La, Ce, Pr, or Nb, and X is either Ge or Si, exhibit  $T_c$ 's of 30-60°C. and may be used in bioprobes. Other oxides of ferrite, garnets, and spinels may be used as bioprobes. For example, substituting (doping) Gd and Al for Fe of  $Y_3Fe_5O_{12}$  garnets provides control of both  $T_c$  and  $4\pi M_s$ . These types of 25 ferromagnetic media are commercially available from, for example, TRAK Ceramics, Hagerstown, MD; Ceramic Magnetics, Fairfield, NJ; and TMC Magnetics, Pine Brook, NJ. Sub-micron size particles may be produced through ball milling, solution-precipitation, or sol-gel processing of these materials.

The heating profile (temperature versus time) of the magnetic material may 30 be determined under a variety of model conditions, for example, as suspensions in water, within cell culture media, agar gel, etc.

In general, a fundamental frequency of several MHz is effective for inductive heating. At such frequencies, however, tissue heating may be problematic. Another frequency range, which results in less tissue heating, is 100 kHz to 500 kHz.

Tissue heating places limitations on the practical amplitude of the magnetic field. A preferred range may be from 100 to 200 Oersteds (Oe), using a constant waveform generator. Difficulties may arise in achieving desired particle heating with many materials when the field falls in this range. The field conditions may also be inadequate because the number of bioprobes attaching to the targeted cells may often be unknown and/or limited by the specifics of cellular biology. The limitation may be overcome by using a pulsed generator and setting the field conditions, field amplitude and pulse characteristics, to levels that heats the bioprobes sufficiently to kill targeted cells without excessive peripheral tissue heating. Thus, under pulsed conditions, peak magnetic fields of up to a few thousand Oersteds (Oe) may be used.

15 Example 2: Synthesis of Bio-Compatible Coatings.

The bioprobe particles selected during Example 1 may be coated with a biocompatible polymer according to a following embodiment of the procedure. Poly (methacrylic acid-*co*-hydroxyethylmethacrylate) as a biocompatible coating material for bioprobes may be synthesized from methacrylic acid and hydroxyethyl methacrylate using free-radical polymerization in the presence of the magnetic particles. To avoid aggregation of the magnetic nanometer sized particles during polymerization, the polymerization may be carried out in a microemulsion environment consisting of water/toluene/sodium bis-2-ethylhexyl sulfosuccinate. The sodium bis-2-ethylhexyl sulfosuccinate acts as an ionic surfactant to make a stabilizing layer around the magnetic particles. Methacrylic acid ( $1.45 \times 10^{-2}$  moles), hydroxyethylmethacrylate ( $5.38 \times 10^{-4}$  moles), *N,N'*-methylene bisacrylamide ( $8.12 \times 10^{-5}$  moles), and 2,2'-azobisisbutyronitrile ( $7.62 \times 10^{-5}$  moles) are added to a mixture of water/toluene/bis-2-ethylhexyl sulfosuccinate ( $0.38/0.47/5.1 \times 10^{-2}$  moles) with magnetic particles. The polymerization may be carried out at 55°C for several hours under nitrogen. Coated bioprobe particles may then be recovered by precipitation in an excess of acetone/ethanol followed by several washings. The

coated bioprobe particles may be kept in vacuum overnight to remove residual monomers.

Example 3: Attachment of the Antibodies to the Particles.

5       The attachment of the antibodies to the coated magnetic particles may be accomplished by use of a small "linker" molecule with differentiated terminals that permit covalent antibody attachment to the biocompatible polymer coating.  
10      Attachment occurs in the Fc region using the electrophilic C-terminal residue of the antibody to react with the nucleophilic portion of the linker. The other terminus of the linker may be activated by UV photolysis. The photoactive end of the linker may react with the polymer coating. Conjugation of the antibodies to the nanoparticles using this technique may proceed in the following manner. The antibody may be covalently conjugated to 4-[p-azidosalicylamido]butylamine (ASBA) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, EDC. The EDC  
15      may activate the C-terminal carboxyl function, making it susceptible to attack by the primary amino group of the ASBA. The antibody, covalently tethered to the linker, may be subjected to photolysis in the presence of the polymer-coated nanoparticles. On exposure to UV, the azido function on the linked ASBA may generate a nitrene *in-situ* that covalently inserts into the polymer coat yielding a nanoparticle that may  
20      be covalently bound to the antibody through the linker.

      If the selected magnetic nanoparticles are not toxic and do not require polymer coating, the antibodies may be attached to the nanoparticles directly. The immunoglobulin G's may be cleaved at the disulfide linkages connecting the two portions of the antibody and purified by chromatography. The fragments, containing  
25      residual sulfhydryl groups, may be conjugated directly to the metal clusters forming coordinate covalent linkages.

Example 4: Efficacy of bioprobes: *In vitro* trials with MCF-7 breast cancer cells.

30      The bioprobes included 50-nm, Fe<sub>3</sub>O<sub>4</sub> particles surrounded by a dextran shell, to which the monoclonal antibody (mAB) for Her-2 was covalently linked.

The particles were suspended in cellular growth media including Modified Eagle's Medium (MEM) containing 10% fetal calf serum, insulin (10 µg/ml), fungizone, glutamine, penicillin, streptomycin, sodium pyruvate and non-essential amino acids, which was then added to cultures of MCF-7 cells. The MCF-7 cells represent an  
5 estrogen receptor-positive human breast carcinoma, and were grown on tissue culture inserts with a 10 mm diameter possessing porous frits with pore sizes of 0.02 or 0.2 µm.

The alternating magnetic field source was an industrial 3.5 kW variable duty radio-frequency generator with a frequency of 740 kHz and time-averaged field  
10 amplitude of 500 Oe (peak field amplitude, 1300Oe). The generator produced pulses with a length of 4.174 ms, at a pulse repetition rate of 121 Hz, to give a duty cycle of 50.6%. The generator provided power to a 14-mm (inner diameter), 5-turn copper solenoid coil into which sample containers were inserted. The average temperature of the media in all samples monitored *in situ* using silicon dioxide  
15 temperature probes resistant to electromagnetic (EM) fields obtained from FISO Technologies Inc., Ste-Foy, Quebec, Canada.

A series of water bath tests was conducted to determine the hyperthermia tolerance of the cell cultures. The purpose of these tests was to obtain "positive control" data on the effects of hyperthermia on the cancer cell cultures and to aid in  
20 the interpretation of experiments using the bioprobe system. Exposing cellular culture media to an alternating magnetic field, without the presence of the bioprosbes, may cause some tissue heating. Such magnetic induction heating is determined by the frequency and field strength of the magnetic field. Thus, to avoid overheating the media with the applied magnetic field, water bath tests were conducted to  
25 provide threshold limits of magnetic induction heating of the media containing cells not receiving the treatment. Table 1 lists results of cell death fraction as a function of exposed temperature and exposure time. The MCF-7 cell line is shown to be unaffected by heat exposure to 42°C for 30 minutes. Thus, for all *in vitro* experiments, the magnetic field strengths were fixed at levels where the average  
30 temperature of the cellular growth media (containing no cells or magnetic fluid) remained at or below 42°C.

**TABLE 1: Result of water bath tests with MCF-7 cells.**

Temperature (°C)	Time of exposure (min.)	% dead cells
37*	N/A	4
42	30	4
43	15	5
46	5	92

\*Represents a control cell culture sample not placed into water bath.

5           Cells intended for treatment were combined with bioprobe containing the Her-2 antibody and incubated for 8 minutes at 20°C, followed by three rinses with growth media, to remove unattached bioprobe. The cell cultures were analyzed before and 6 hours after the 20-minute treatments with the alternating magnetic field (AMF). The fraction of dead cells for the targeted sample (T1) and control samples  
10          (C1-C3) are presented in Table 2. In the targeted sample,  $91\% \pm 5\%$  ( $n = 7$ ) of the MCF-7 cells were killed. Of the cells killed, about 70% cells were lysed by the treatment, as measured by spectrophotometric analysis of cytoplasmic lactate dehydrogenase (LDH), an enzyme produced by living cells. The remaining approximately 20% underwent apoptosis, as measured using a commercial  
15          fluorescent apoptosis-staining assay. The kill rates for the targeted cells are significantly higher than baseline death and apoptotic rates of  $4\% \pm 1\%$  in all controls (Table 2). Control groups include: 1) cells receiving no exposure to either the AMF or bioprobe (Sample C1); 2) cells exposed to the bioprobe alone (Sample C2); and 3) cells exposed to the AMF alone (Sample C3). Similar baseline deaths of  
20          all controls confirm that neither the AMF alone, nor only the presence of the bioprobe, is toxic to the cells. Higher than normal cell death occurs only when the AMF is applied after the bioprobe have attached to a cell.

**TABLE 2: In Vitro Results with MCF-7 Cells.**

SAMPLE	TREATMENT	% DEAD CELLS (N=7)
C1	NO BIOPROBES, NO AMF	4 ± 1
C2	BIOPROBES, NO AMF	5 ± 1
C3	NO BIOPROBES; AMF	4 ± 1
T1	BIOPROBES WITH AMF	91 ± 5

**Example 5: Selectivity of bioprobes: *In vitro* trials with SK-CO-1 colon cancer cells.**

5       For the *in vitro* studies, the MCF-7 human breast cancer cells were chosen because their Her-2 expression was sufficient for detection, but was not expressed in extraordinarily high amounts as is the case with the more aggressive MDA-MB-231 (human breast carcinoma cells known to significantly over express Her-2) cell line. As a control, cultures of SK-CO-1 cells (human colon adenocarcinoma cells known 10 to be Her-2 negative) were chosen to provide a reasonable model to challenge the effectiveness and selectivity of the bioprobe system.

15      To investigate the selectivity of the bioprobes, SK-CO-1 human colon cancer cells known to be Her-2 negative were treated in the same manner. For this group, rates of apoptosis were 13% for cells treated with an alternating magnetic field (AMF) and bioprobes, 18% for cells receiving no AMF and no bioprobes (represents normal death rate), 10% for cells exposed only to an AMF, and 8% for cells exposed to only bioprobes. These results are summarized in Table 3. Thus, no significant differences were seen in the death rates of SK-CO-1 cells when used with any or all components of the invention, demonstrating selective tumoricidal activity for the 20 MCF-7 breast cells.

**TABLE 3: In Vitro Results with SK-CO-1 Cells.**

SAMPLE	TREATMENT	% DEAD CELLS
SK-CO-1	NO BIOPROBES, NO AMF	18
SK-CO-1	BIOPROBES, NO AMF	8
SK-CO-1	NO BIOPROBES; AMF	13
T1 (MCF-7)	BIOPROBES WITH AMF	91 ± 5 (N=7)

**Example 6: Bioprobe targeting MUC-1 receptor in breast cancer.**

The target on the breast cancer cells may be MUC-1 (Human epithelial mucin, CD277) marker. MUC-1 is highly expressed in many human adenocarcinomas including 80% of breast cancers and is associated with poor prognosis. The ligand on the bioprobe may be an antibody which targets the MUC-1 receptor. Commercial monoclonal mouse anti-human MUC-1 antibodies may be used in preliminary *in vitro* studies, from such sources as Chemicon International, Temecula, CA; and Zymed Laboratories, South San Francisco, California. The MUC-1 bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-human MUC-1 is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

*In vitro* studies demonstrate dose response and proof of killing of cancer cells above the level of the controls. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-human MUC-1 antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the tumor targeting and potency. The use of fragments, humanized antibody, or peptides creates a ligand that avoids immunogenic response in humans.

The MUC-1 receptor is considered a truly tumor specific antigen because although it is on normal cells, its aberrant glycosylation on tumors creates new epitopes for targeting. The extracellular domain of MUC-1 is shed into the blood stream which makes the selection of the targeting ligand a very important 5 component in this example. The MUC-1 targeting ligand may be selected to avoid the shed portion of the extracellular domain of MUC-1 in the bloodstream. This may result in reducing delivery problems and dilution of the treatment by bioprobes binding to the shed receptor. The MUC-1 targeting ligand recognizes an epitope of MUC-1 which remains on the cancer cell after a portion of the extracellular domain 10 is shed. This choice of ligand allows the bioprobe to specifically and selectively target breast cancer cells.

Example 7: Bioprobe targeting non-small cell lung cancer, VEGFR.

The bioprobes may be designed to target many different kinds of cancer. A 15 target on non-small cell lung cancer cells may be VEGFR (vascular endothelial growth factor receptor). marker. VEGFR may also be a viable target for many other types of solid cancer tumors. VEGFR has been implicated in tumor induced angiogenesis, or blood vessel growth which has been shown to play an important role in the development of many solid tumors. Angiogenesis is induced by solid 20 tumors to provide nutrients to enable growth. The bioprobe therapy may target tumor-induced angiogenesis and prevent the blood vessel growth needed by a tumor. Since there is little, if any, blood vessel growth in a healthy adult, the targeting of VEGFR should allow for safer treatment options.

Commercial monoclonal mouse anti-human VEGFR antibodies may be used 25 in preliminary *in vitro* studies, from such sources as Lab Vision Corp., Fremont, CA. The VEGFR bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-human VEGFR is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may 30 be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

*In vitro* studies demonstrate dose response and proof of killing of cancer cells above the level of the controls. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-human VEGFR antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the tumor targeting and potency. The use of fragments, humanized antibody, or peptides creates a ligand that avoids immunogenic response in humans.

10

Example 8: Bioprobe targeting immune system conditions, CD52.

A target rheumatoid arthritis may be cluster designation/differentiation CD52 antigen. CD52 may also be a viable target for other immune system diseases such as multiple sclerosis. For diseases of the patient's immune system, the bioprobe therapy may target the T or B cells of the immune system. Typically these cells are involved in an immune response. T lymphocytes play a central role in initiating and controlling the immune response and the molecular basis for immune recognition and the way the body distinguishes invaders from its own tissues is becoming known. This may bring new insight into a wide range of severe diseases, including rheumatoid arthritis, multiple sclerosis and many others, all of which appear to result in whole or in part from a breakdown of the normal mechanisms of self-tolerance. The damage which occurs in these autoimmune diseases is analogous to the rejection process which occurs after transplantation of a foreign organ. Adults also have active mechanisms for maintaining self-tolerance which are controlled by a complex symphony of interactions between different parts of the immune system. Bioprobes with antibodies to CD52 can destroy lymphocytes and disrupt the immune system at the most fundamental level. Unlike cancer therapy, it may not be the aim to kill all the target cells, but just remove enough to allow a respite for the tissue under attack. The hope was that when the immune system regenerated over the subsequent months, the vicious cycle of tissue injury, resulting in fresh immune stimulation, would have subsided sufficiently for the natural control mechanisms to

restore a healthy balance. It may be necessary to monitor T cells during the period of regeneration in order to direct them into a more appropriate pathway of self-tolerance.

Commercial monoclonal rat anti-human CD52 antibodies may be used in preliminary *in vitro* studies, from such sources as Biosource International, Camerillo, CA. The CD52 bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-human CD52 is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-human CD52 antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency. The use of fragments, humanized antibody, or peptides creates a ligand that avoids immunogenic response in humans.

20 Example 9: Bioprobe targeting bacterial conditions.

The bioprobes may be designed to target different kinds of pathogen-borne conditions. A pathogen may be any disease producing agent such as, for example, a bacterium, a virus, a microorganism, a fungus, and a parasite. One example of a target for a bacterial pathogen is *Escherichia coli*. *Escherichia coli* is an emerging cause of foodborne illness. Currently, an estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Most illness has been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. *E. coli* O157:H7 is one of hundreds of strains of the bacterium *Escherichia coli*. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness. In this embodiment of the

bioprobe therapy, the target is the bacterium itself. The pathogen may be in the blood stream, tissue, or cells. The bioprobe therapy may target and kill the bacteria in the patient. It will also be possible to target infected cells with an appropriate cell surface marker on the cells.

5        Commercial monoclonal goat anti-*Escherichia coli* O157:H7 antibodies may be used in preliminary *in vitro* studies, from such sources as Accurate Chemical and Scientific, Westbury, NY. The *Escherichia coli* targeted bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The goat anti-*Escherichia coli* O157:H7 is a whole antibody and the Fc portion may be linked to the magnetic particle or the  
10      dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

15      *In vitro* studies demonstrate dose response and proof of killing above the level of the controls. The AMF device will be appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal goat anti- *Escherichia coli* O157:H7 antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered  
20      properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

Example 10: Bioprobe targeting viral conditions, Hepatitis B.

25      The bioprobes may be designed to target different kinds of pathogen-borne conditions such as a virus. An example of a target for a viral pathogen is Hepatitis B. Hepatitis B is a serious disease caused by a virus that attacks the liver. The virus, which is called hepatitis B virus (HBV), can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death. In this particular embodiment of bioprobe therapy, the target is the virus itself. The pathogen may be  
30      in the blood stream, tissue, or cells.

Commercial monoclonal rabbit anti-Hepatitis B antibodies may be used in preliminary *in vitro* studies, from such sources as Chemicon International, Temecula, CA. The Hepatitis B bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The rabbit anti-Hepatitis B antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

The AMF device is appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-Hepatitis B antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

Example 11: Bioprobe targeting viral conditions, HIV.

Another example of a target for a viral pathogen is human immunodeficiency virus, HIV, the cause of AIDS. Although it has long been possible to detect the presence of HIV, scientists have struggled to distinguish infected cells from healthy ones. In this example, the target is associated with the infected cell. For example, when a cell produces HIV, a protein called CTLA4 appears on the cell surface. In this particular example, the CTLA4 protein is used as the bioprobe target. Infected cells tend to hoard copies of the virus inside instead of releasing them steadily after infection. CTLA4 only migrates to the cell's outer surface when the hoarded virus is ready to be released. In this application of bioprobe therapy, the target is the CTLA4 protein marker on infected cells. The cells may be in the blood stream, tissue, or cells. For example, based on a diagnostic test, a patient would use bioprobe therapy when the CTLA4 protein is expressed on the cell surface and the HIV virus is ready to launch. These cells can be destroyed before they have the chance to spread the virus.

- Commercial monoclonal rabbit-anti-human CTLA4 antibodies may be obtained from such sources as Chemicon International, Temecula, CA, and used in preliminary *in vitro* studies. The CTLA-4 bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The monoclonal rabbit-anti-human CTLA4 is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.
- 10        The AMF device is be appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal rabbit-anti-human CTLA4 antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.
- 15

Example 12: Bioprobe targeting parasitic conditions.

The bioprobes may be designed to target different kinds of parasitic conditions. One example of a target for a parasitic pathogen is *Trypanosoma cruzi*. *Trypanosoma cruzi* is the American form of a parasite that causes Chagas' disease and is transmitted via blood sucking Reduvid bugs. Acute phase symptoms include fever, malaise, lymphatic system abnormalities, and enlargement of the spleen and liver. Fatal pathologies include parasitic infection of the heart or meninges. Chronic manifestations include myocardial damage and arrhythmias, megaesophagus, and megacolon. Diagnosis is by finding the parasite in the blood. In this application of bioprobe therapy, the target is the pathogen itself. The pathogen may be in the blood stream, tissue, or cells. The bioprobe therapy targets and kills the parasite in the patient. It is also possible to target infected cells with an appropriate cell surface marker on the cells.

Commercial monoclonal mouse anti-*Trypanosoma cruzi* antibodies may be used in preliminary *in vitro* studies, from such sources as Accurate Chemical and Scientific, Westbury, NY. The *Trypanosoma cruzi* targeted bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-*Trypanosoma cruzi* antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

The AMF device will be appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-*Trypanosoma cruzi* antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

Example 13: Bioprobe targeting fungal conditions.

The bioprobes may be designed to target different kinds fungal conditions. One example of a target for a fungal pathogen is be Rhizomucor. Rhizomucor, also known as the *Zygomycetous fungus*, is reported to be allergenic, and may cause mucorosis in immune compromised individuals. Rhizomucor occupies a biological niche similar to Mucor. It is often linked to occupational allergy, and may cause mucorosis in immune-compromised individuals. The sites of infection are the lung, nasal sinus, brain, eye and skin and infection may have multiple sites.

In this application of bioprobe therapy, the target is the pathogen itself. The pathogen may be in the blood stream, tissue, or cells. The bioprobe therapy targets and kills the fungus in the patient. It may also be possible to target infected cells with an appropriate cell surface marker on the cells.

A commercial mouse anti-Rhizomucor fungus monoclonal antibody may be used in preliminary *in vitro* studies, from such sources as DAKO Corp., Carpinteria,

CA . The Rhizomucor targeted bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-Rhizomucor fungus antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

5                   The AMF device will be appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-Rhizomucor fungus antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, 10 fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

15                  Example 14: Bioprobe targeting toxins in the patient.

The bioprobes may be designed to target different kinds of toxin in the patient. A toxin is defined as any poison produced by an organism including, but not limited to, bacterial toxins, plant toxins, insect toxin, animal toxins, and man-made toxins. A bacterial toxin target may be, for example, one of Cholera toxin, 20 Diphteria toxin, and Clostridium botulinus toxin. An insect toxin may be, for example, bee venom. An animal toxin may be, for example, the snake toxin, *Crotalus durissus terrificus* venom.

In this example of an application of bioprobe therapy, the target is the toxin itself. The toxin may be in the blood stream, tissue, or cells. The bioprobe therapy 25 may target and destroy the toxin in the patient. In the particular example described here, the target is the bacterial toxin, Cholera.

Commercial monoclonal rabbit anti-Cholera toxin antibodies may be used in preliminary *in vitro* studies, from such sources as Accurate Chemical and Scientific, Westbury, NY. The Cholera -targeted bioprobes may be formed from 50 nm 30 dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The rabbit anti-Cholera toxin antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran

coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

The AMF device is appropriate to the location of treatment. The targeted  
5 bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal rabbit anti-Cholera toxin antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the  
10 targeting and potency.

Example 15: Bioprobe targeting an undesirable target.

The bioprobes may be designed to target an undesirable target in a patient. An undesirable target is a target that may be associated with a disease or an  
15 undesirable condition, but is also present in the normal condition. For example, the target may be elevated or otherwise altered in the disease or undesirable state. The bioprobe may have an affinity for the undesirable target or biological molecules from pathways related to the undesirable target or it may have an affinity for a cell marker or markers associated with the undesirable target.

20 In this particular example, in arteriosclerosis, a target is low density lipoprotein (LDL). A marker in LDL is apolipoprotein B which can be targeted for bioprobe therapy. Atherosclerosis is a type of arteriosclerosis involving deposits of fatty substances, cholesterol, cellular waste products, calcium and fibrin (a clotting material in the blood) in the inner lining of an artery.

25 Atherosclerosis can affect the arteries of the brain, heart, kidneys, other vital organs, and the arms and legs. When atherosclerosis develops in the arteries that supply the brain (carotid arteries), a stroke may occur; when it develops in the arteries that supply the heart (coronary arteries), a heart attack may occur.

30 In the United States and most other Western countries, atherosclerosis is the leading cause of illness and death. Despite significant medical advances, coronary artery disease (which results from atherosclerosis and causes heart attacks) and

atherosclerotic stroke are responsible for more deaths than all other causes combined. Atherosclerosis is a slow, progressive disease that may start in childhood.

5 In this example of an application of bioprobe therapy, the target is apolipoprotein B. The bioprobe therapy may be used to target, denature, or otherwise deactivate apolipoprotein B found in plaques in the patient. This may prevent or mitigate the deposition of excess LDL or treat the condition.

Commercial monoclonal goat anti-apolipoprotein B antibodies may be used in preliminary *in vitro* studies, from such sources as Chemicon International, 10 Temecula, CA. The apolipoprotein B -targeted bioprosbes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The goat anti-apolipoprotein B antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody 15 may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

The AMF device will be appropriate to the location of treatment. The targeted bioprosbes may be further refined by producing Fab and scFV fragments of a monoclonal goat anti-apolipoprotein B antibody, fragments from an antibody 20 library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

25 Example 16: Bioprobe associated with reaction to an organ transplanted into the patient.

The bioprosbes may be designed to target cells, tissue, or biological molecules involved in a reaction to an organ transplanted into the patient. This application is similar to diseases of the patient's immune system. The bioprobe therapy may target 30 the T or B cells of the immune system. Typically these cells are involved in an immune response. T lymphocytes play a central role in initiating and controlling the

immune response and the molecular basis for immune recognition and the way the body distinguishes invaders from its own tissues is becoming known. The damage which occurs in the rejection process which occurs after transplantation of a foreign organ is analogous to autoimmune diseases. Bioprobes which can destroy  
5 lymphocytes and disrupt the immune system at the most fundamental level can be used to treat organ transplant rejection. Unlike cancer therapy, it may not be the aim to kill all the target cells, but just remove enough to allow a respite for the tissue under attack. The hope was that when the immune system regenerated over the subsequent months, the vicious cycle of tissue injury, resulting in fresh immune stimulation would have subsided sufficiently for the natural control mechanisms to restore a healthy balance. It may be necessary to monitor T cells during the period of regeneration in order to direct them into a more appropriate pathway of self-tolerance.  
10

In this particular example, the bioprobe target is the CD52 antigen on T cells. CD52 antigen has been associated with kidney and bone marrow transplantation. Commercial monoclonal rat anti-human CD52 antibodies may be used in preliminary *in vitro* studies, from such sources as Biosource International, Camerillo, CA. The CD52 bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-human CD52 is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.  
20

The AMF device will be appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a monoclonal mouse anti-human CD52 antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.  
25  
30

Example 17: Bioprobe targeting non-cancerous diseased tissue.

The bioproses may be designed to target cells, tissue, or biological molecules related to a non-cancerous disease. One particular example of a target related to a non-cancerous disease is B protein and its deposits associated with Alzheimer's disease. Bioproses designed to specifically bond to lipoproteins that transport amyloids in the vascular system can be used to target, denature, or otherwise deactivate these lipoproteins when exposed to AMF treatment. This may prevent or mitigate the deposition of excess amyloids in various organs of humans or animals (amyloidosis) or treat the condition. Similarly bioproses can be used to target, denature or otherwise deactivate amyloids directly to prevent or treat the deposition of excess amyloids.

A common form of amyloidosis is Alzheimer's disease. Although not fully understood, the accumulation of amyloid protein in the brain to form plaque (amyloid fibrils) is part of the pathology. Bioproses which are designed to specifically bond to the lipoprotein, amyloid B protein, associated with Alzheimer's disease, as they are transported in the vascular system or after they are deposited in the brain, can be used to target, denature or otherwise deactivate these lipoproteins. The residual amyloid proteins may be absorbed or otherwise removed from the body.

The bioprobe target is amyloid B protein. Commercial monoclonal rabbit anti-amyloid B protein antibodies may be used in preliminary *in vitro* studies, from such sources as Accurate Chemical and Scientific, Westbury, NY. The-amyloid B protein targeted bioproses may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The rabbit anti-amyloid B protein antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

The AMF device will be appropriate to the location of treatment. The targeted bioproses may be further refined by producing Fab and scFV fragments of a monoclonal rabbit anti-amyloid B protein antibody, fragments from an antibody

library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

5

Example 18: Bioprobe targeting a proteinaceous pathogen.

The bioprobes may be designed to target cells, tissue, or biological molecules related to a proteinaceous pathogen. An example of a target is prion protein 3F4 associated with prion diseases known as transmissible spongiform encephalopathies.

10 Novel infectious particles, termed prions, composed largely and perhaps solely of a single protein, are the likely causative agents of a group of transmissible spongiform encephalopathies that produce lethal decline of cognitive and motor function. Evidence indicates that the responsible protein arrives at a pathogenic state by misfolding from a normal form that has ubiquitous tissue distribution.

15 Bioprobes designed to specifically bond to prions such as Prion protein 3F4 can be used to target, denature, or otherwise deactivate these prions when exposed to AMF treatment. This may prevent, mitigate, or treat the condition. Commercial monoclonal mouse anti-human prion protein 3F4 antibodies may be used in preliminary *in vitro* studies, from such sources as DAKO Corp., Carpinteria, CA.

20 The prion protein 3F4-targeted bioprobes may be formed from 50 nm dextran coated Fe<sub>3</sub>O<sub>4</sub> particles. The mouse anti-human prion protein 3F4 antibody is a whole antibody and the Fc portion may be linked to the magnetic particle or the dextran coating through several different techniques. A linking agent, such as BS<sup>3</sup>, available from Pierce, Rockford, IL, may be used with silanization or the antibody may be 25 biotinylated and attached to a streptavidin-containing, 50 nm dextran coated particle.

25 The AMF device is appropriate to the location of treatment. The targeted bioprobes may be further refined by producing Fab and scFV fragments of a mouse anti-human prion protein 3F4 antibody, fragments from an antibody library, humanization of the antibody, or production of peptides. Antibodies, fragments, and peptides may be engineered with altered properties such as antigen binding affinity,

molecular architecture, and specific receptor epitope targeting to enhance the targeting and potency.

Example 19: Bioprobe targeting a hormone-related disease.

5       The bioproses may be designed to target cells, tissue, or biological molecules related to a hormone disease. In one particular example, growth hormone is a peptide anterior pituitary hormone essential for growth. The hypothalamus maintains homeostatic levels of growth hormone. Cells under the action of growth hormone increase in size (hypertrophy) and number (hyperplasia). Growth hormone  
10      also causes increase in bone length and thickness by deposition of cartilage at the ends of bones. During adolescence, sex hormones cause replacement of cartilage by bone, halting further bone growth even though growth hormone is still present. Too much growth hormone can cause gigantism. Bioproses designed to target human growth hormone can be used to denature, or otherwise deactivate excess hormone  
15      when exposed to AMF treatment. Commercial monoclonal rabbit anti-human growth hormone antibodies may be used in preliminary *in vitro* studies, from such sources as Accurate Chemical and Scientific, Westbury, NY.

          In another example, overproduction of the female hormone estrogen may increase the risk for breast cancer. The number of new cancer cells increases proportionally to the amount of overexpression by the estrogen precursor--and these effects may be prevented, mitigated, or treated using bioprobe therapy. Bioproses designed to target estrogen can be used to denature, or otherwise deactivate excess hormone when exposed to AMF treatment. Commercial monoclonal rabbit anti-estrogen antibodies may be used in preliminary *in vitro* studies, from such sources as  
20      US Biological, Swampscott, MA.  
25

Example 20: Extracorporeal Approach to Bioprobe Therapy.

          In extracorporeal hyperthermal therapy, bioproses may be used to lyse, denature, or otherwise destroy the desired target cells or antigens by circulating the  
30      blood outside of the body, exposing it to AMF, and returning it to the body. The bioproses may be introduced into the vascular circulating system or into the blood

circulating outside of the body. For example, to target bioprobe/target complexes that are carried primarily in the blood serum or plasma, the blood serum or plasma may be extracorporeally separated from the other blood components, exposed to AMF to destroy the target, and recombined with the other blood components before 5 returning the blood to the body. The bioprobes may be introduced into the vascular circulating system, the blood circulating outside of the body, or the blood serum or plasma after it is separated. In another embodiment, the bioprobes may be contained in a vessel or column through which the blood circulating outside of the body or the blood serum or plasma flows. The vessel or column may be exposed to AMF to 10 destroy the targeted cells or antigens before returning the blood to the body.

The advantages of exposing bioprobes to AMF, and heating outside of the body include the ability to heat to higher temperatures and/or heat more rapidly to enhance efficacy while minimizing heating and damage to surrounding body tissue, and reducing exposure of the body to AMF energy. If the bioprobes are introduced 15 into the blood circulating outside the body or the blood serum or plasma after being extracted from the body, advantages include i) no introduction of ferromagnetic bioprobes directly into the body and ii) higher concentrations of bioprobes to targeted cells or antigens. In addition, the bulk blood or blood serum/plasma can be cooled externally by a variety of means as it is being heated by the bioprobes, which 20 will not mitigate the localized heating by the bioprobe, or after the heating, but before returning it to the body.

Example 21: Pretargeting Approach to Bioprobe Therapy.

A pretargeting therapy approach to the bioprobes may be used to achieve 25 higher concentration of bioprobes at the targeted site. Pretargeting therapy may also allow for higher doses of ligand or multiple doses of the ligand to be given to the patient and may allow for more time for the ligand to localize at the predetermined target. In pretargeting therapy, the targeting ligand is infused into the body separately from the magnetic material composition and allowed to accumulate at the 30 target site. For example, the ligand may be comprised of an antigen-binding portion to bind the antigen at the target site and a high affinity binding site for a small

molecule. A clearing agent may be infused to remove unbound ligand circulating in the bloodstream. The magnetic material composition linked to the small molecule is added. The magnetic material travels to the targeted site and attaches to the ligand bound at the treatment site. For example, a streptavidin conjugated antibody  
5 may be used with a biotinylated magnetic particle composition. The antibody is typically administered to the patient prior to the magnetic particle and allowed to localize at the predetermined target. Since the pretargeting antibody is typically non-toxic, high doses may be given. A clearing agent may or may not be used to remove unbound antibody depending on the residence time in the body. The  
10 biotinylated magnetic particle composition is administered to the patient at an appropriate time for bioprobe therapy. The streptavidin conjugated antibody and biotinylated magnetic particle may combine in the patient's body prior to bioprobe therapy. The strong biotin-streptavidin association is used to bind the magnetic particle to the antibody in the patient.

15

Example 22: Temperature monitoring during treatment.

In a treatment cycle, the bioproses **210** may be exposed to and become attached to metastatic cells in the treatment area. Pole pieces **204** may be chosen to provide treatment in a selected treatment area, for example, approximately 6 inches  
20 in diameter. The patient **105** is typically placed on the treatment bed **206**, and the bed controller **108** is typically adjusted to place the targeted tissue at the region of maximum field strength between the poles **204**. The pulse width, duty cycle, and peak amplitude of the AMF and the treatment time may be adjusted. Several temperature probes, for example, four temperature probes, may be inserted into the  
25 treatment volume. One probe may be located centrally and the other three may be inserted into tissues, within the treatment area that are heated by the AMF. As the tissue heats under the applied AMF, the temperature probes **354** send data to the probe monitor **352**. The monitor sends the temperature data via the feedback loop **324** to the RF generator controller **356**. Monitoring may be continuous, for example  
30 at a data rate of 10 samples per second and the duty of the RF generator **318** may be adjusted once per second based on individual, 10 sample averages from each probe.

If none of the probes 354 sense a temperature greater than the preset limit, the RF generator 318 may be permitted to operate at the original settings. If one or more of the probes 354 senses a temperature over the preset threshold, the controller 356 may send a command to reduce at least one of the duty cycle, the PRF, the  
5 magnitude of the magnetic field. This process continues until the treatment is completed.

Example 23: Preliminary temperature monitoring to optimize treatment.

Using the temperature probes described in Example 22 or an infrared thermal  
10 monitor, a practical map of the field strength can be attained by operating at a reduced power level and monitoring the tissue temperature in and around the desired treatment area. This information may be used to adjust the operating parameters of the auxiliary coils. Any or all of the following auxiliary coil operating parameters may be adjusted manually and/or automatically, based upon the tissue temperature  
15 map; i. Auxiliary coil peak current, ii. Auxiliary coil pulse width, iii Auxiliary coil current phase, relative to primary coil current. When the temperature difference between the tissue in the treatment area and the surrounding tissue is maximized, then the auxiliary coils' operating parameters have been optimized for the given auxiliary coils' positions. This preliminary adjustment may be performed using a  
20 suitable tissue model thereby reducing the patient's exposure and discomfort.

While the above description of the invention has been presented in terms of a human patient, it is appreciated that the invention may also be applicable to treating disease in other mammals.

As noted above, the present invention is applicable to a magnetic material  
25 composition, a system and method of thermotherapy for the treatment of at least cancerous tissue, non-cancerous diseased tissue and undesirable tissue. The present invention should not be considered limited to the particular examples described above, but rather should be understood to cover all aspects of the invention as fairly set out in the attached claims. Various modifications, equivalent processes, as well  
30 as numerous structures to which the present invention may be applicable will be readily apparent to those skilled in the art to which the present invention is directed

upon review of the present specification. The claims are intended to cover such modifications and devices.

1. A method for treating a patient, comprising:
  - 5 administering a magnetic material composition to at least a portion of the patient, the magnetic material composition comprising at least one, single domain, magnetic particle attached to a ligand specific to a predetermined target in the patient; and
  - applying an alternating magnetic field to the magnetic material composition combined with the predetermined target to inductively heat the magnetic material composition.
- 10 2. The method as recited in claim 1, wherein the predetermined target is associated with a cancer.
- 15 3. The method as recited in claim 2, wherein the predetermined target is one of a) a member of vascular endothelial growth factor receptor (VEGFR) family, b) a member of carcinoembryonic antigen (CEA) family, c) an anti-idiotypic mAB, d) a ganglioside mimic, e) a member of cluster designation/differentiation (CD) antigen family, f) a member of epidermal growth factor receptor (EGFR) family, g) a cellular adhesion molecule, h) a member of MUC-type mucin family, i) a cancer antigen (CA), j) a matrix metalloproteinase, k) a glycoprotein antigen, l) a melanoma associated antigen (MAA), m) a proteolytic enzyme, n) a calmodulin, o) a member of tumor necrosis factor (TNF) receptor family, p) a angiogenic marker, q) a 20 melanoma antigen recognized by T cells (MART) antigen, r) a member of melanoma antigen encoding gene (MAGE) family, s) a prostate membrane specific antigen (PMSA), t) a small cell lung carcinoma antigen (SCLCA), u) a proliferating cell nuclear antigen 10 (PC10), v) pS2, w) a T/Tn antigen, x) a hormone receptor, y) a tumor suppressor gene antigen, z) a cell cycle regulator antigen, aa) an oncogene 25 antigen, bb) an oncogene receptor antigen, cc) a proliferation marker, dd) a proteinase involved in degradation of extracellular matrix, ee) a malignant transformation related factor, ff) an apoptosis-related factor gg) a human carcinoma antigen and any combination of a) through gg).

4. The method as recited in claim 3 wherein the ganglioside mimic is GD3.
  5. The method as recited in claim 3, wherein the epidermal growth factor receptor (EGFR) includes at least one of Her-1, Her-2, Her-3, Her-4 and any combination thereof.
  6. The method as recited in claim 3, wherein the cluster designation/differentiation antigen includes at least one of CD20 antigen, CD22 antigen, CD52 antigen, CD64 antigen, CD31 antigen, CD33 antigen and any combination thereof.
- 10 7. The method as recited in claim 3, wherein the cellular adhesion molecule is epithelial cellular adhesion molecule (Ep-CAM) antigen.
8. The method as recited in claim 3, wherein the MUC-type mucin includes at least one of polymorphic epithelial mucin (PEM), MUC-1 and any combination thereof.
- 15 9. The method as recited in claim 3, wherein the cancer antigen is at least one of CA125, CA19-9, CA15-3 (breast tumor antigen), CA242 and any combination thereof.
10. The method as recited in claim 3, wherein the matrix metalloproteinase is matrix metalloproteinase 2 (MMP-2).
- 20 11. The method as recited in claim 3, wherein the glycoprotein antigen is epithelial associated glycoprotein (HMFGI antigen).
12. The method as recited in claim 3, wherein the proteolytic enzyme is at least one of cathepsin G, and cathepsin D, cathepsin L, cathepsin B, cathepsin C, cathepsin S and any combination thereof.

13. The method as recited in claim 3, wherein the calmodulin is calponin.

14. The method as recited in claim 3, wherein the tumor necrosis factor receptor is a TRAIL Receptor-1 protein.

15. The method as recited in claim 3, wherein the angiogenic marker is at least one of vascular endothelial growth factor receptor (VEGFR), Integrin  $\alpha v\beta 3$  and any combination thereof.

16. The method as recited in claim 2, further comprising:  
applying an alternating magnetic field to a region of the patient containing the cancer, wherein the alternating magnetic field is applied using a device comprising:  
a magnetic generator having a core defining at least part of a magnetic circuit, two poles of the core defining a gap therebetween, a magnetic field passing between two poles, the gap being of sufficient size to receive a portion of the patient containing the cancer cells; and  
15 a power supply coupled to provide energy to the magnetic generator so that the magnetic field passing between the two poles alternates at a frequency of about 1 kHz or more.

17. A method according to claim 16, wherein the magnetic material composition inductively heats to a sufficient temperature to induce cancer cell death via necrosis, apoptosis or another mechanism.

18. A method according to claim 16, further comprising applying the alternating magnetic field to a region of the patient containing cancer tissue and to a region of the patient adjacent the region containing the cancer tissue.

19. A method according to claim 16, further comprising applying a static magnetic field to a region of the patient containing cancer tissue to aid in localizing the magnetic material composition to the region containing the cancer tissue.

20. A method according to claim 16, further comprising monitoring at least one physical characteristic of at least one of cancer tissue and non-cancerous disease material.

5 21. A method according to claim 16, further comprising pulsing the alternating magnetic field while applying the alternating magnetic field and exposing the patient to at least two pulses of the alternating magnetic field while applying the alternating magnetic field.

10 22. A method according to claim 16, wherein the alternating magnetic field is applied to the patient over a treatment duration, and applying the alternating magnetic field includes pulsing the alternating magnetic field to have a pulse length less than the treatment duration and applying at least two pulses of alternating magnetic field to the patient.

15 23. The method as recited in claim 16, wherein administering the magnetic material composition further comprises administering the material to a specific area of a patient's body via an administration technique being at least one of injecting the composition intravenously, injecting the composition intraperitoneally, injecting the composition intravascularly, injecting the composition intramuscularly, injecting the composition subcutaneously, applying the composition topically, applying the composition via a wash, lavage or rinse perisurgically, inhaling the composition, orally ingesting the composition and rectally inserting the composition.

20 24. A method according to claim 16, wherein a magnetic field generator generates the alternating magnetic field and wherein the alternating magnetic field has a strength in the range of about 10 Oersteds (Oe) to about 10,000 Oe proximate the magnetic field generator.

25 25. A method according to claim 16, wherein applying the alternating magnetic field includes modulating the alternating magnetic field and modulating

the magnetic field includes applying a modulation having one of a sinusoidal envelope, a triangular envelope, a square wave envelope, a trapezoidal envelope and a sawtooth envelope.

26. A method according to claim 16, wherein the magnetic material  
5 composition includes a biocompatible coating material covering at least part of the magnetic particle.

27. The method as recited in claim 1, wherein the predetermined target is an antigen associated with a disease of a patient's immune system, the disease being one of rheumatoid arthritis, vasculitis and multiple sclerosis.

10 28. The method as recited in claim 27, wherein the predetermined target is a cluster designation/differentiation antigen.

29. The method as recited in claim 1, wherein the predetermined target is a virus, the predetermined target being one of Epstein-Barr virus (EBV), Hepatitis virus, human immunodeficiency virus (HIV) and Herpes virus.

15 30. The method as recited in claim 1, wherein the predetermined target is one of an undesirable material and associated with an undesirable material, the predetermined target being one of undesired adipose tissue, gastric inhibitory polypeptide receptor, low density lipoprotein (LDL) and clotted blood.

20 31. The method as recited in claim 1, wherein the predetermined target is associated with a reaction to an organ transplanted into the patient, the predetermined target being at least one of human T cell receptor CD3 antigen, CD4 antigen, CD 8 antigen, CD18 antigen, CD52 antigen and CD154 antigen.

32. The method as recited in claim 1, wherein the predetermined target is non-cancerous disease material and associated with non-cancerous disease material,

the predetermined target being one of a non-cancerous disease deposit and a non-cancerous disease precursor deposit.

33. The method as recited in claim 32, wherein the predetermined target is associated with Alzheimer's disease and wherein the predetermined target is one of an amyloid protein and an apolipoprotein.  
5

34. The method as recited in claim 32, wherein the predetermined target is associated with an HIV infected cell and wherein the predetermined target is CTLA4 protein.

35. The method as recited in claim 1, wherein administering the magnetic material composition further comprises administering the material to a specific area of a patient's body via an administration technique being at least one of injecting the composition intravenously, injecting the composition intraperitoneally, injecting the composition intravascularly, injecting the composition intramuscularly, injecting the composition subcutaneously, applying the composition topically, applying the composition via a wash, lavage or rinse perisurgically, inhaling the composition, 10 orally ingesting the composition and rectally inserting the composition.  
15

36. The method as recited in claim 1, wherein administering the magnetic material composition further comprises:

administering the ligand and the magnetic particle to the patient separately;  
20 and  
combining the ligand with the magnetic particle in the patient's body.

37. The method as recited in claim 36, wherein administering the magnetic material composition further comprises:

administering the ligand, having one of a binder and a receptor, to the  
25 patient;

administering the magnetic particle, having the other of the binder and the receptor to the patient; and

combining the ligand with the magnetic particle by uniting the binder and receptor in the patient's body.

5       38.     The method as recited in claim 37, wherein the binder is biotinylated and the receptor is an avidin.

39.     The method as recited in claim 36, wherein administering the magnetic material composition further comprises:

administering a bispecific ligand to the patient;

10      administering a magnetic material conjugated to a target molecule to the patient; and

combining the magnetic particle with the ligand in the patient's body.

40.     The method as recited in claim 1, wherein applying the alternating magnetic field includes applying the alternating magnetic field when the magnetic material composition is outside the patient's body.

15      41.     The method as recited in claim 40, wherein administering comprises providing the magnetic material composition within the patient's body and extracting the magnetic material composition from the patient's body after attaching to the target.

20      42.     The method recited in claim 40, further comprising removing, after applying the alternating magnetic field, the magnetic material composition from extracted bodily materials and prior to returning the extracted bodily materials to the patient's body.

25      43.     The method as recited in claim 40, wherein administering comprises extracorporeal administration of the magnetic material composition into a patient's bodily materials.

44. The method as recited in claim 43, wherein extracorporeal administration comprises:

extracting bodily materials from the patient;

administering the magnetic material composition to the extracted bodily materials; and

exposing the bodily materials to the alternating magnetic field.

45. The method as recited in claim 40, wherein the extracted bodily materials are returned to the patient's body after exposure to the alternating magnetic field.

10 46. The method as recited in claim 40, wherein the extracted bodily materials are returned to the patient's body before exposure to the magnetic field.

47. The method as recited in claim 40, wherein the extracted bodily materials include at least one of blood and blood products extracted from the patient.

15 48. The method as recited in claim 1, further comprising detecting at least one location of accumulation of the magnetic material composition within the patient's body.

20 49. The method as recited in claim 48, wherein detecting the at least one location of accumulation of the magnetic material includes using at least one of magnetic resonance imaging (MRI) and a superconducting quantum interference device (SQUID) after administering the composition to the patient and prior to application of the alternating magnetic field.

50. The method as recited in claim 49, further comprising determining an amount of the magnetic material composition taken up by the targeted material by direct or indirect detection of the particles via at least one of MRI and SQUID.

51. The method as recited in claim 50, wherein the determined amount of magnetic material composition taken up by the targeted material is used to calculate a condition and duration of the alternating magnetic field to be applied.

52. The method as recited in claim 1, further comprising inducing a desired pathological effect by inductively heating the magnetic material causing one of necrosis, apoptosis and deactivation of disease material in a targeted region.

53. The method as recited in claim 1, further comprising applying the alternating magnetic field to a region of the patient containing the predetermined target and to a region of the patient adjacent to the region containing the predetermined target.

54. The method as recited in claim 1, further comprising localizing the magnetic material composition to a region containing the predetermined target by applying a d.c. component of the alternating magnetic field to the region of the patient containing the predetermined target.

15 55. The method as recited in claim 1, further comprising monitoring at least one physical characteristic of a portion of the patient, the at least one physical characteristic being one of tissue temperature and tissue impedance.

20 56. The method as recited in claim 1, wherein the alternating magnetic field is applied to the patient during a treatment duration, and applying the alternating magnetic field comprises pulsing the alternating magnetic field with a pulse length less than the treatment duration via applying at least two pulses of the alternating magnetic field to the patient.

25 57. The method as recited in claim 1, further comprising generating with a magnetic field generator an alternating magnetic field having a strength in the range of about 10 Oersteds (Oe) to about 10,000 Oe proximate the magnetic field generator.

58. The method as recited in claim 1, wherein applying the alternating magnetic field comprises modulating the alternating magnetic field, wherein modulating the magnetic field further comprises applying a modulation having one of a sinusoidal envelope, a triangular envelope, a square wave envelope, a trapezoidal envelope and a sawtooth envelope.

59. The method as recited in claim 1, wherein applying the alternating magnetic field comprises:

providing and positioning auxiliary coils to influence a magnetic field pattern generated by a primary coil, and further comprises:

10 driving at least one of the primary and auxiliary coils with a d.c. component to influence the magnetic field pattern.

60. The method as recited in claim 59, wherein the d.c. component has a magnitude sufficient to influence the magnetic field pattern by causing the alternating magnetic field to have at least one of a unidirectional nature with varying intensity and a time-averaged, non-zero value.

61. The method as recited in claim 59, further comprising driving the auxiliary coils in series with the primary coil.

62. The method as recited in claim 59, further comprising driving the auxiliary coils in parallel with the primary coil.

20 63. The method as recited in claim 59, further comprising electrically matching the auxiliary coils with a phase relationship so as to maximize the field strength in a specific area and minimizing the field strength in areas which may experience detrimental effects from the alternating magnetic field.

25 64. The method as recited in claim 59, further comprising influencing the magnetic field pattern to separate the patient from an electric field component associated with the magnetic field, so as to reduce dielectric heating of the patient.

65. The method as recited in claim 1, wherein applying the alternating magnetic field further comprises providing a primary coil as a core-less coil.

66. The method as recited in claim 1, wherein applying the alternating magnetic field comprises applying the alternating magnetic field using only a  
5 primary coil.

67. The method as recited in claim 1, wherein applying the alternating magnetic field includes generating the alternating magnetic field with a magnetic field generator and further comprises cooling the magnetic field generator with liquid.

10 68. The method as recited in claim 67, further comprising cooling a core of the magnetic field generator with liquid.

15 69. The method as recited in claim 1, wherein applying the alternating magnetic field includes driving a primary coil formed of at least first and second coils, the first and second coils disposed proximate respective first and second pole pieces of a magnetic core.

70. The method as recited in claim 1, wherein applying the alternating magnetic field further includes driving a plurality of coils located along a magnetic core.

71. A magnetic material composition, comprising:  
20 a particle having magnetic properties and forming a single magnetic domain;  
a biocompatible coating material for the particle; and  
a ligand selective to at least one disease material marker associated with  
disease material, the ligand being one of bound to an uncoated portion of the  
particle, bound to a coated portion of the particle, bound to the particle and partially  
25 covered by the coating and intercalated into the coating.

72. The magnetic material composition of claim 71, wherein the ligand is one of a molecule and a combination of molecules selective to at least one specific targeted disease material or disease material marker.

5 73. A magnetic material composition of claim 71, wherein the disease material marker is specific to one of breast cancer, metastatic cancer related to breast cancer and primary cancer of the breast.

74. A magnetic particle composition of claim 71, wherein the biocompatible coating material is biodegradable.

10 75. A magnetic particle composition of claim 71, wherein the particle has a size of no more than 250 nm in at least one dimension.

76. A magnetic material composition of claim 71, wherein the particle, the coating and the ligand are suspended in a biologically compatible fluid.

15 77. A magnetic material composition of claim 71, wherein the magnetic particle is one of ferromagnetic, antiferromagnetic, ferrimagnetic, antiferrimagnetic and superparamagnetic.

78. A magnetic material composition of claim 71, wherein the magnetic particle has a Curie temperature in the range of about 40 °C to about 150 °C.

20 79. A magnetic material composition of claim 71, wherein the magnetic particle is formed of a biocompatible material, the surface of the magnetic particle forming the biocompatible coating.

80. A magnetic material composition of claim 71, wherein the biocompatible coating material is one of an organic material, an inorganic material, and a combination of an inorganic material and an organic material.

81. A magnetic material composition of claim 80, wherein the organic material is at least one of a synthetic material and a biological material.

82. A magnetic material composition of claim 81, wherein the synthetic material is a polymer, a copolymer or a combination thereof.

5 83. A magnetic material composition of claim 81, wherein the synthetic material includes at least one of i) a polymer, ii) a copolymer, and iii) a polymer blend formed from a polymer based on at least one of acrylates, siloxanes, styrenes, acetates, alkylene glycols, alkylenes, alkylene oxides, parylene, lactic acid and glycolic acid.

10 84. A magnetic material composition of claim 81, wherein the synthetic material includes one of i) a hydrogel polymer, ii) a histidine-containing polymer, iii) a surfactant and a combination of at least one of i) – iii).

15 85. A magnetic material composition of claim 81, wherein the biological material includes one of i) a polysaccharide, ii) a polyaminoacid, iii) a protein, iv) a lipid, v) a glycerol, vi) a fatty acid and a combination including at least one of i) – vi).

20 86. A magnetic material composition of claim 85, wherein the polysaccharide includes one of a) a heparin, b) heparin sulfate, c) chondroitin sulfate, d) chitin, e) chitosan, f) cellulose, g) dextran, h) alginate, i) starch, j) saccharide, k) carbohydrate, l) glycosaminoglycan and a combination of any of a) – l).

87. A magnetic material composition of claim 85, wherein the protein includes one of a) an extracellular matrix protein, b) proteoglycan, c) glycoprotein, d) albumin, e) peptide, f) gelatin and a combination of any of a) – f).

88. A magnetic material composition of claim 81, wherein the biological material is a transfection agent configured to enhance uptake by the disease material.

89. A magnetic material composition of claim 88, wherein the transfection agent includes one of i) a vector, ii) a prion, iii) a polyaminoacid, iv) a cationic liposome, v) an amphiphile, vi) a non-liposomal lipid and a combination of any of i) – vi).

90. A magnetic material composition of claim 89, wherein the vector includes one of a) a plasmid, b) a virus, c) a phage, d) a viron, e) a viral coat and a combination of any of a) –e).

10 91. A magnetic material composition of claim 89, wherein the polyaminoacid is a poly L-lysine.

92. A magnetic material composition of claim 71, wherein the disease material target includes one of a) a member of vascular endothelial growth factor receptor (VEGFR) family, b) a member of carcinoembryonic antigen (CEA) family, c) an anti-idiotypic mAB, d) a ganglioside mimic, e) a member of cluster designation/differentiation (CD) antigen family, f) a member of epidermal growth factor receptor (EGFR) family, g) a cellular adhesion molecule, h) a member of MUC-type mucin family, i) a cancer antigen (CA), j) a matrix metalloproteinase, k) a glycoprotein antigen, l) a melanoma associated antigen (MAA), m) a proteolytic enzyme, n) a calmodulin, o) a member of tumor necrosis factor (TNF) receptor family, p) a angiogenic marker, q) a melanoma antigen recognized by T cells (MART) antigen, r) a member of melanoma antigen encoding gene (MAGE) family, s) a prostate membrane specific antigen (PMSA), t) a small cell lung carcinoma antigen (SCLCA), u) a proliferating cell nuclear antigen 10 (PC10), v) pS2, w) a T/Tn antigen, x) a hormone receptor, y) a tumor suppressor gene antigen, z) a cell cycle regulator antigen, aa) an oncogene antigen, bb) an oncogene receptor antigen, cc) a proliferation marker, dd) a proteinase involved in degradation of extracellular

matrix, ee) a malignant transformation related factor, ff) an apoptosis-related factor gg) a human carcinoma antigen, hh) DF3 antigen, ii) 4F2 antigen, jj) MFGM antigen and any combination of a) through jj).

5        93. A magnetic material composition of claim 92, wherein the MUC-type mucin includes at least one of polymorphic epithelial mucin (PEM), MUC-1 and any combination thereof.

94. A magnetic material composition of claim 92, wherein the epidermal growth factor receptor (EGFR) includes at least one of Her-1, Her-2, Her-3, Her-4 and any combination thereof.

10      95. A magnetic material composition of claim 81, wherein the synthetic material is a transfection agent.

96. A magnetic material composition of claim 95, wherein the transfection agent includes one of i) a non-lipid cationic polymer, ii) a dendrimer, iii) polyethyleneimine and a combination of any of i)- iii).

15      97. A magnetic material composition of claim 72, wherein the molecule includes one of i) a saccharide, ii) a carbohydrate, iii) a glycan, iv) a protein, v) a peptide, vi) an antibody, vii) an antibody fragment, viii) a receptor, ix) a Cluster Designation/Differentiation (CD) marker, x) a cytokine, xi) a chemokine, xii) a nucleotide, xiii) a lipid, xiv) a steroid, xv) a neurotransmitter, xvi) a lectin, xvii) an imprinted polymer, xviii) an oncogene, xix) an oncogene receptor and a combination of any of i) – xix).

20      98. A magnetic material composition of claim 97, wherein the protein includes one of a) a membrane protein, b) a proteoglycan, c) a cell surface protein, d) a glycoprotein and a combination of any of a) – d).

99. A magnetic material composition of claim 97, wherein the antibody includes one of a) a polyclonal antibody, b) a monoclonal antibody, c) a chimeric antibody, d) a humanized antibody, e) a human antibody, f) a recombinant antibody, g) a bispecific antibody, h) an antibody fragment, i) a recombinant single chain antibody fragment and a combination of any of a) - i)

5  
100. A magnetic material composition of claim 97, wherein the nucleotide includes a) a complete nucleotide, b) a nucleotide fragment, c) a complementary nucleotide and a combination of a) – c).

101. A magnetic material composition of claim 97, wherein the lipid includes one of a) phospholipid, b) glycolipid and a combination of a) and b).

102. A magnetic material composition of claim 80, wherein the inorganic material includes one of i) a metal, ii) a metal alloy, iii) a ceramic, iv) an oxide of a Group IV element and a combination of any of i) – iv).

15  
103. A magnetic material composition of claim 102, wherein the ceramic includes one of a) hydroxyapatite, b) silicon carbide, c) carboxylate, d) sulfonate, e) phosphate, f) ferrite, g) phosphonate and a combination of any of a) – g).

104. A magnetic material composition of claim 71, further comprising a linking agent linking between the ligand and the magnetic particle or between the ligand and the biocompatible coating material.

20  
105. A magnetic material composition of claim 104, wherein the linking agent links to at least one of i) an amine group, ii) a sulphydryl group, iii) a carbohydrate group, iv) a carboxyl group, v) a hydroxyl group and a combination of any of i) - v).

25  
106. A magnetic material composition of claim 104, wherein the linking agent is comprised of at least one of i) homobifunctional crosslinkers, ii)

heterobifunctional crosslinkers, iii) aldehydes, iv) homotrifunctional crosslinkers, v) heterotrifunctional crosslinkers and a combination of any of i) - v).

107. A device for treating disease material by interacting with magnetic particles targeted to disease material in a patient, the device comprising:

- 5           a magnetic generator having a core defining at least part of a magnetic circuit, two poles of the core defining a gap therebetween, a magnetic field passing between two poles, the gap being of sufficient size to receive a portion of the patient containing the disease material; and
- 10           a power supply coupled to provide energy to the magnetic generator so that the magnetic field passing between the two poles alternates at a frequency of about 1 kHz or more.

108. A device for treating disease material according to claim 107, wherein the level of the alternating magnetic field is sufficient to heat the magnetic particles to an effective temperature for treating the disease material.

- 15           109. A device for treating disease material according to claim 107, wherein the poles of the core define a gap sufficiently large to receive the portion of the patient containing the disease material, the poles of the core are formed by removable pole pieces of selected shape, the pole pieces being in an adjustable relationship with each other so that a size of the gap is adjustable.

- 20           110. A device for treating disease material according to claim 107, wherein the fundamental frequency of the alternating magnetic field is in the range of about 1 kHz to about 1 GHz.

- 25           111. A device for treating disease material according to claim 107, wherein the alternating magnetic field has one of a sinusoidal fundamental waveform and a non-sinusoidal fundamental waveform.

112. A device for treating disease material according to claim 107, wherein the alternating magnetic field has a fundamental waveform that is one of i) square wave, ii) trapezoidal, iii) sawtooth and iv) triangular.

113. A device for treating disease material according to claim 107,  
5 wherein the alternating magnetic field is amplitude modulated with a pulse width of 1 nanosecond to 600 seconds and a pulse repetition frequency of about 0.0017 Hz to about 1000 MHz.

114. A device for treating disease material according to claim 107,  
wherein the alternating magnetic field is modulated with a duty cycle of less than  
10 100 %.

115. A device for treating disease material according to claim 107,  
wherein at least one of the fundamental frequency and the amplitude of the alternating magnetic field is modulated.

116. A device for treating disease material according to claim 115,  
15 wherein the alternating magnetic field is amplitude modulated with one of i) sinusoidal modulation, ii) a triangular modulation, iii) a square wave modulation, iv) a trapezoidal modulation and v) a sawtooth modulation and a combination of any of i) – v).

117. A device for treating disease material according to claim 115,  
20 wherein the alternating magnetic field is repetitively modulated having a pulse repetition frequency, the pulse repetition frequency being modulated.

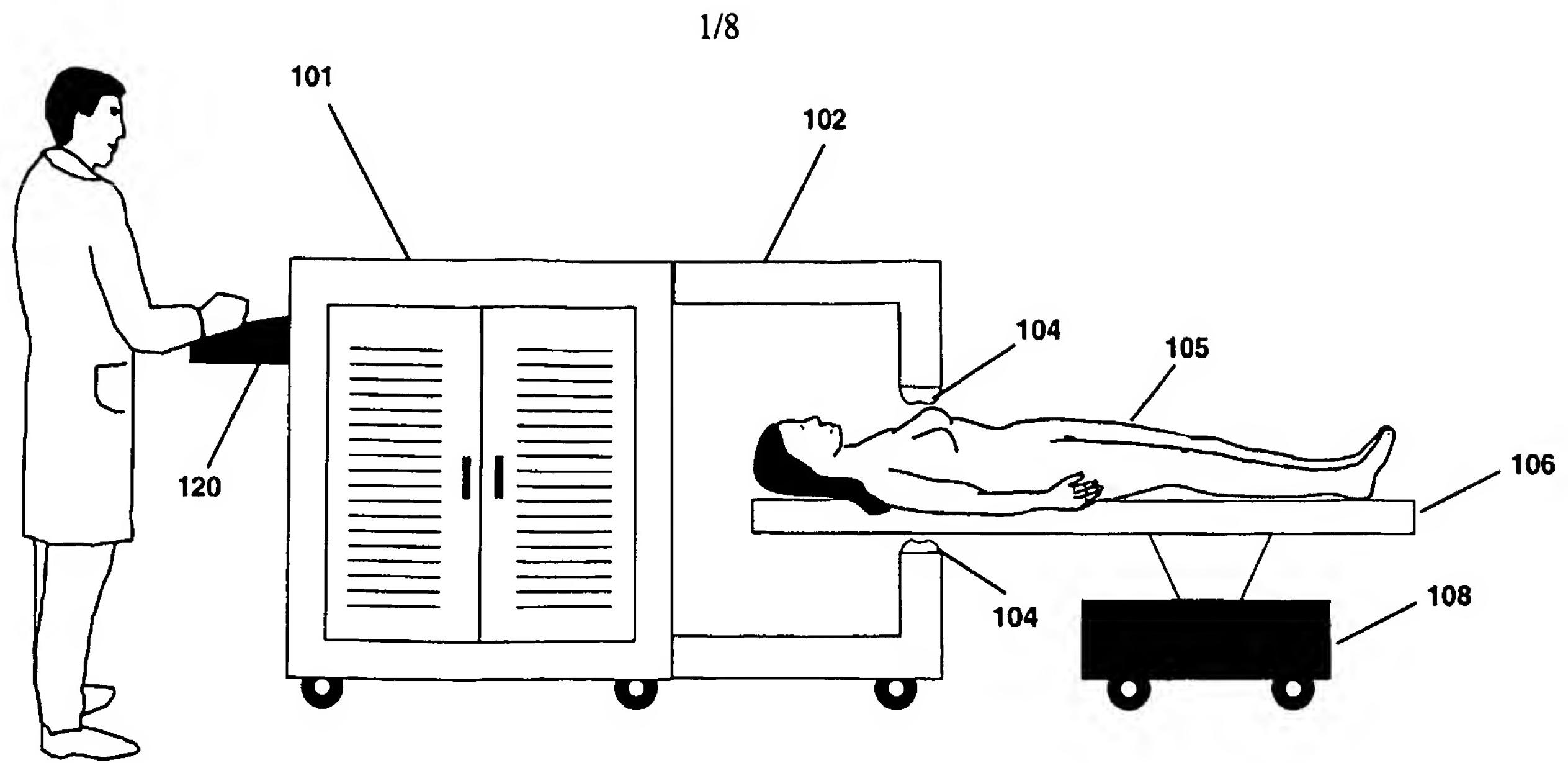
118. A device for treating disease material according to claim 107,  
wherein the alternating magnetic field between the poles of the core has a strength in the range from about 10 to about 10,000 Oersteds (Oe).

119. A device for treating disease material according to claim 107, further comprising a detector to monitor a physical characteristic of the portion of the patient being treated, and a feedback circuit to control the power supply in response to the monitored physical characteristic.

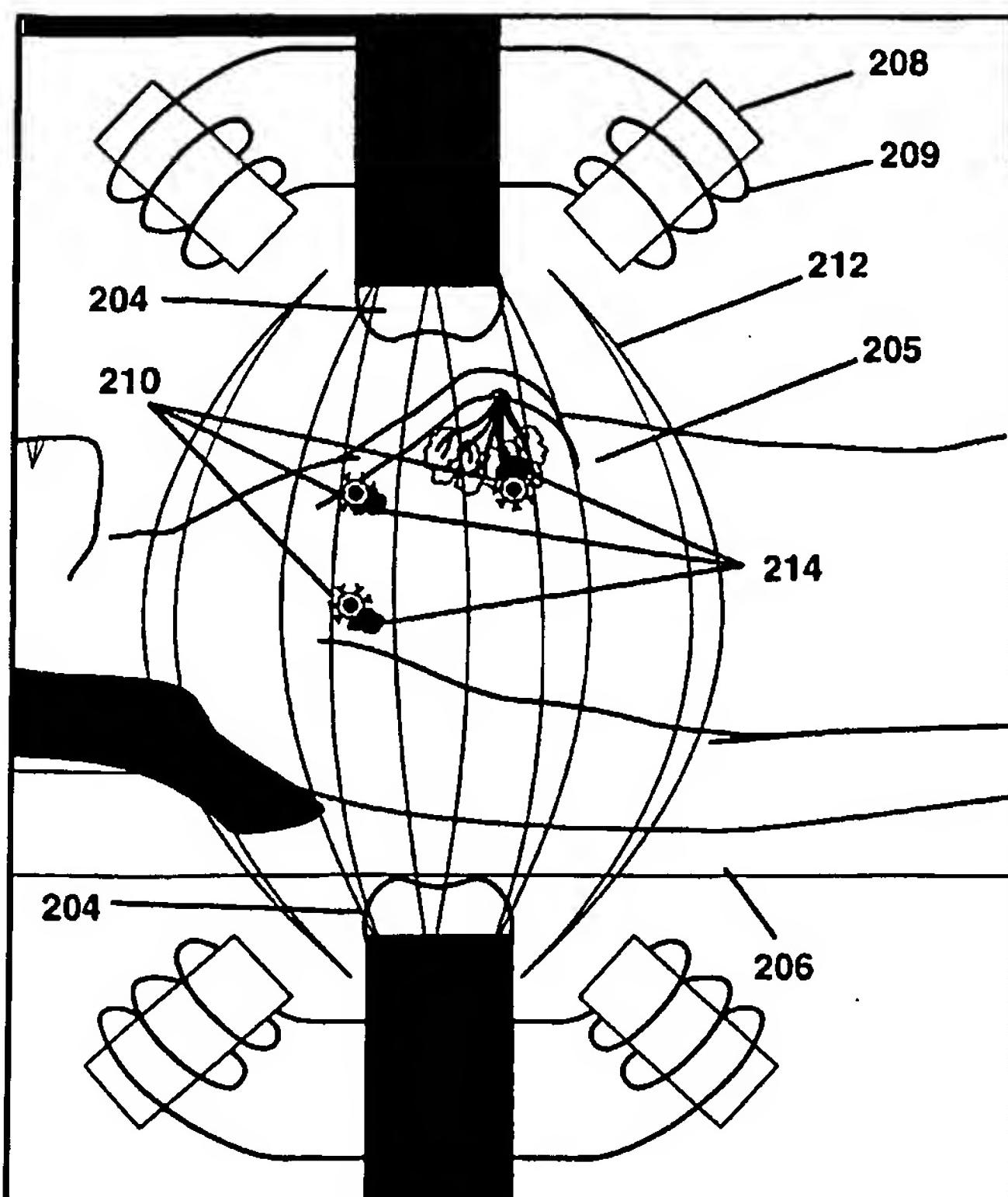
5

120. A device for treating disease material according to claim 119, wherein the detector is one of a temperature monitor and an impedance monitor.

121. A device for treating disease material according to claim 107, wherein the magnetic generator is liquid cooled.



**Figure 1.**



**Figure 2.**

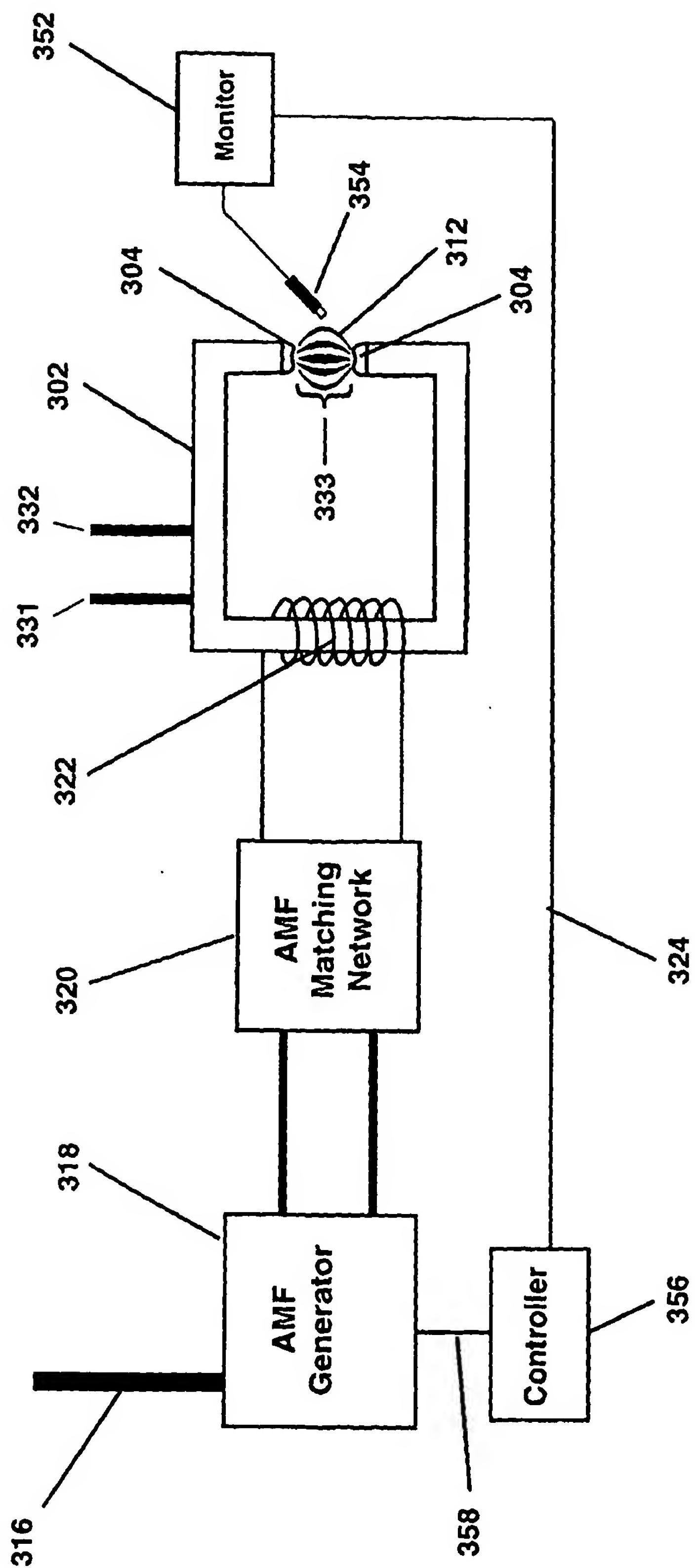
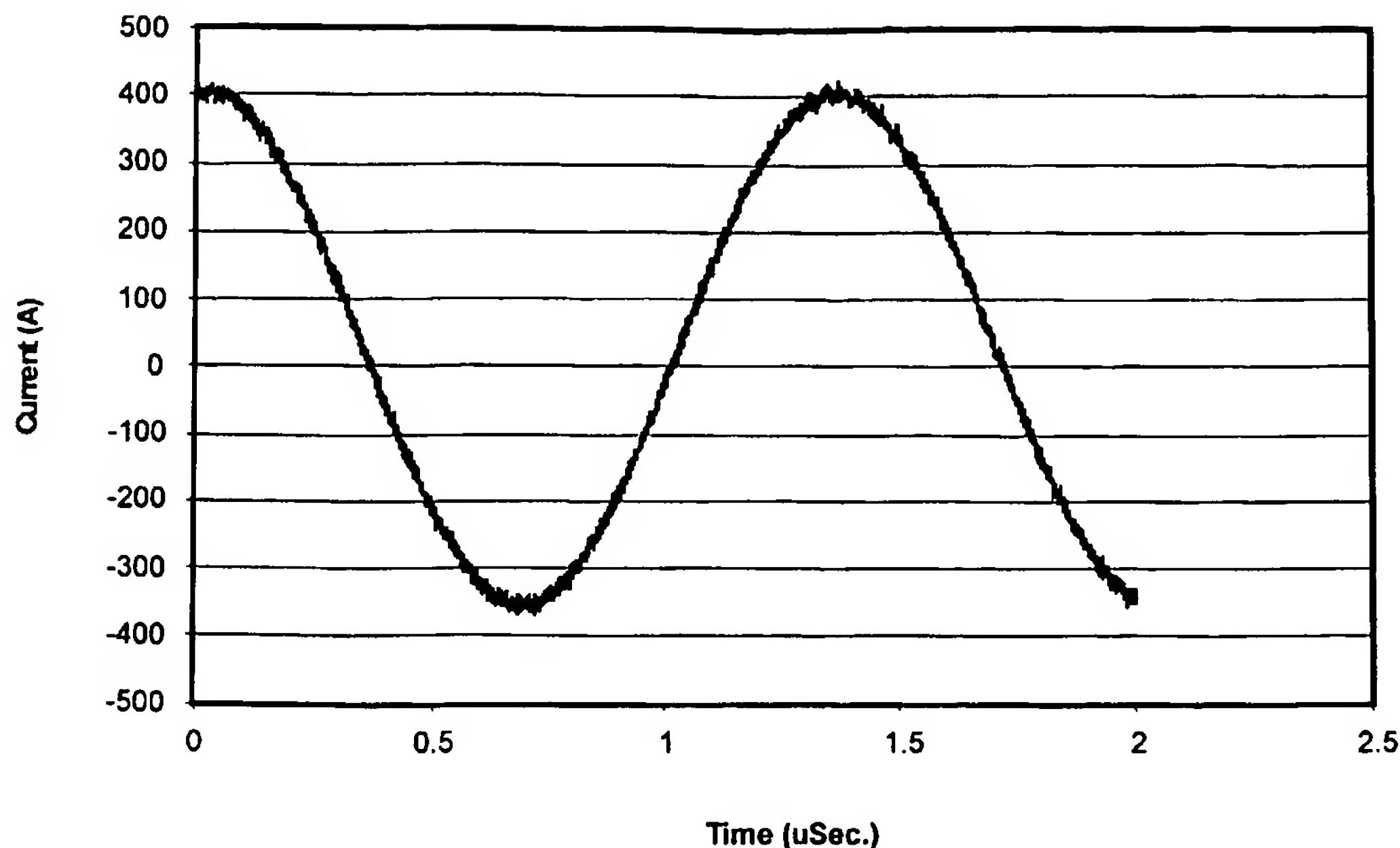
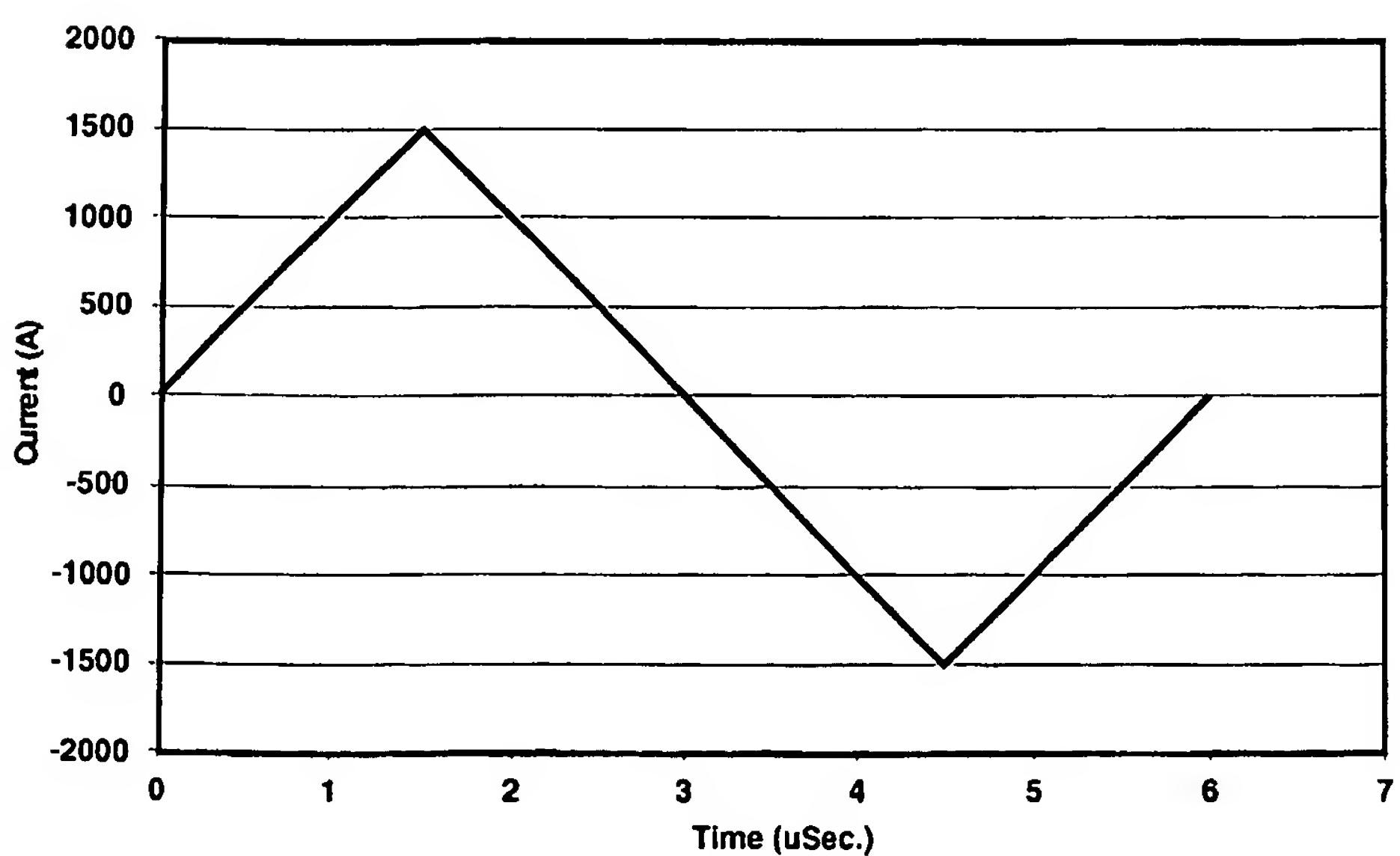
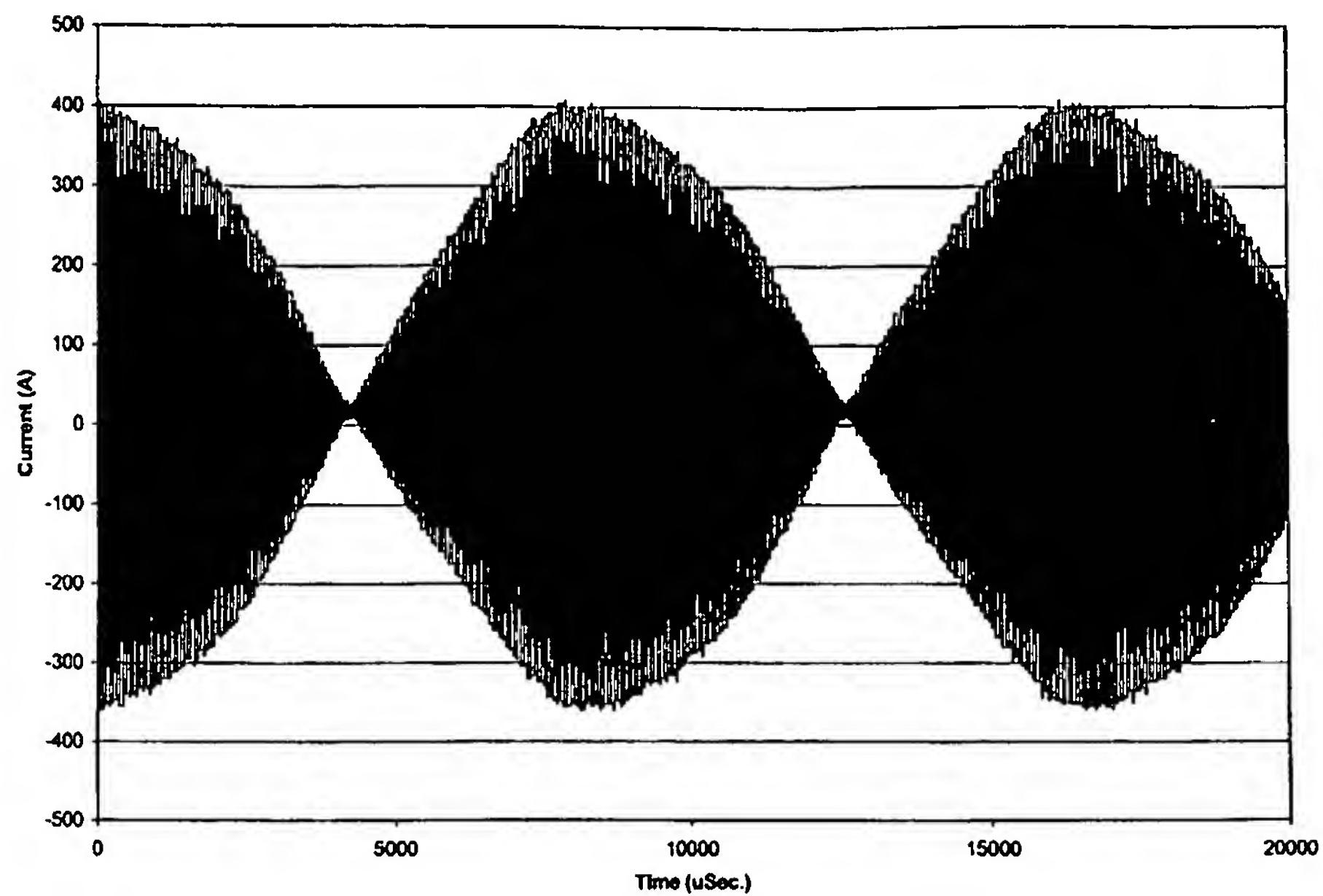


Figure 3.

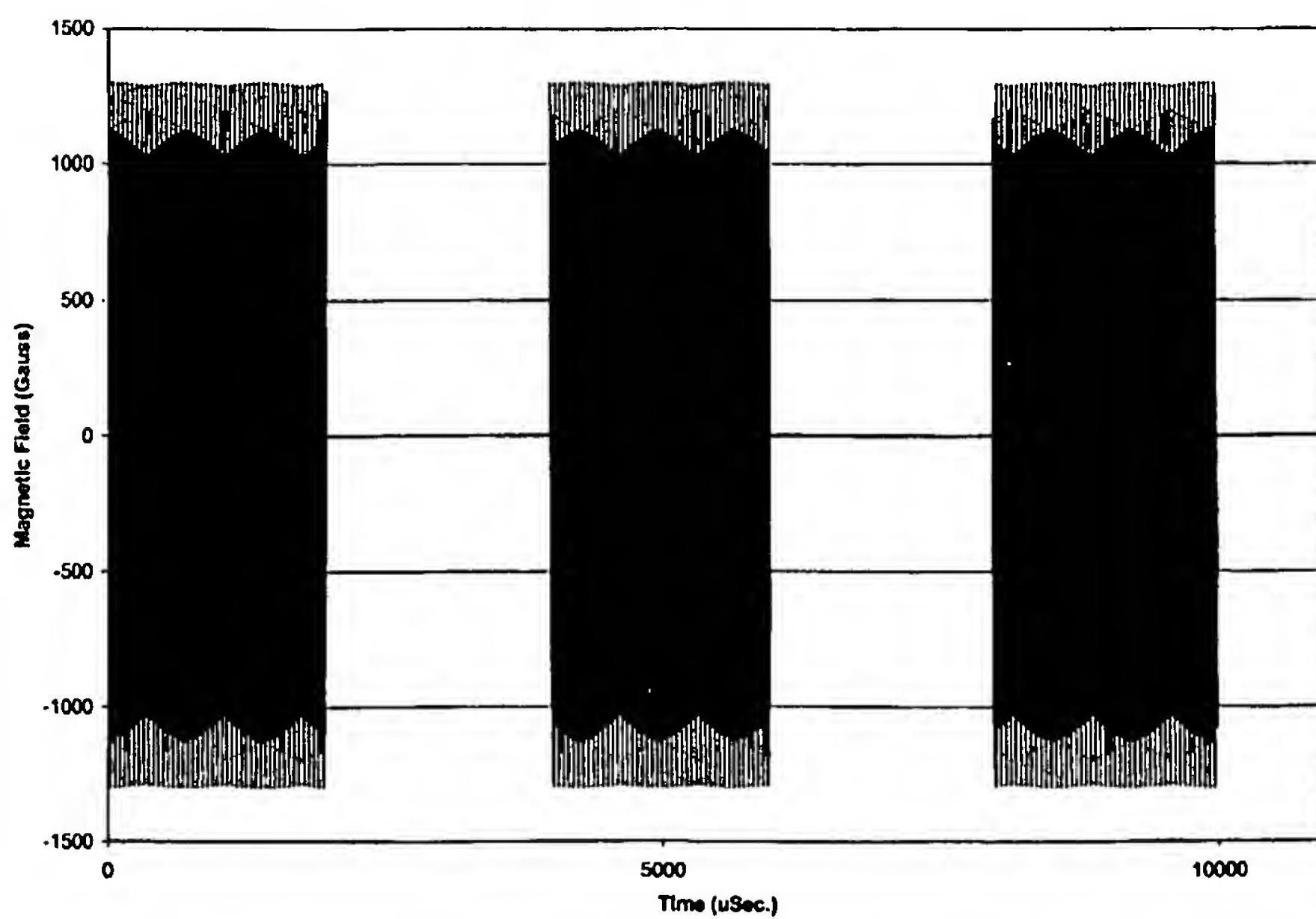
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**Figure 4a.****Figure 4b.**

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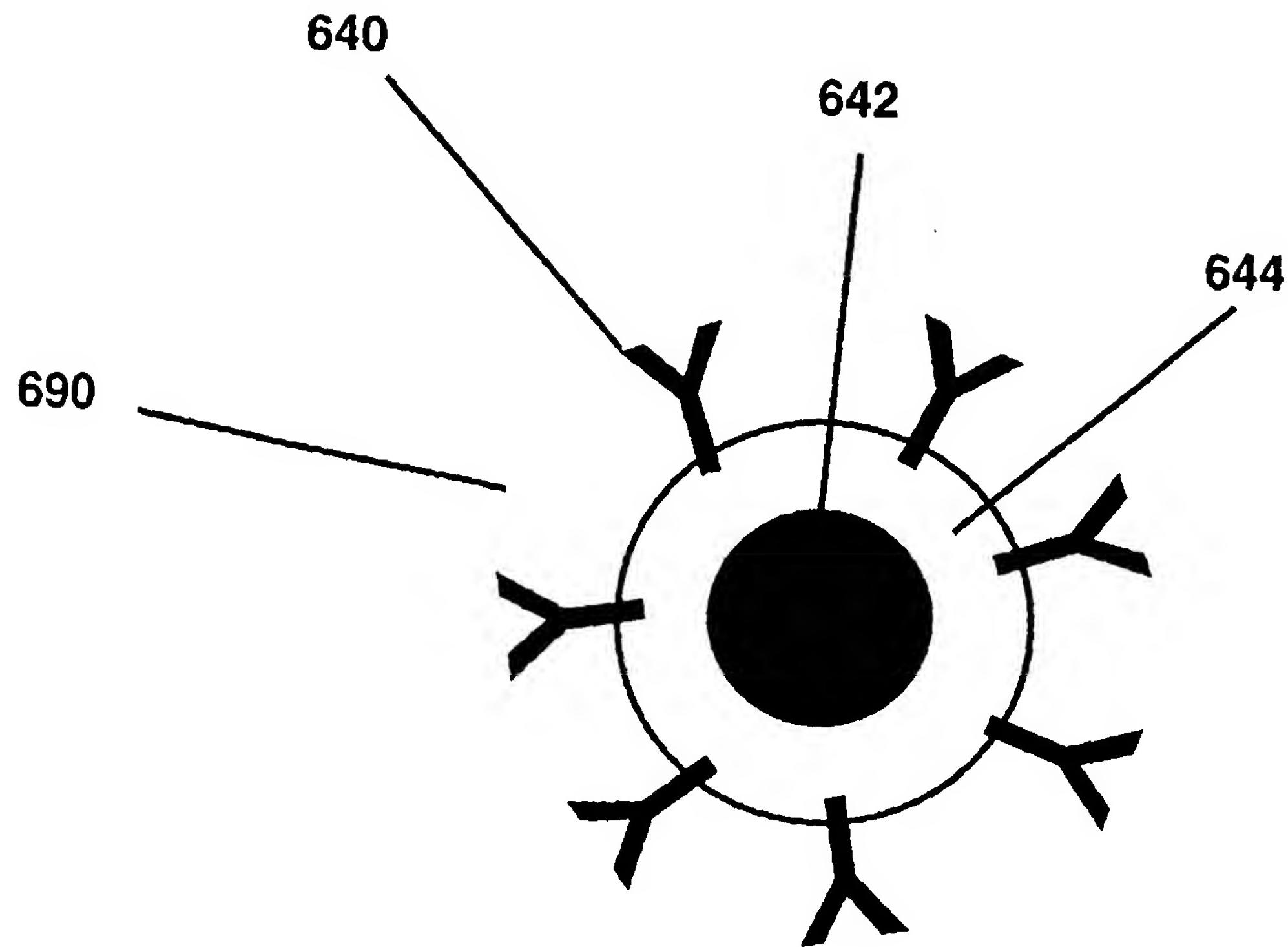


**Figure 5a.**

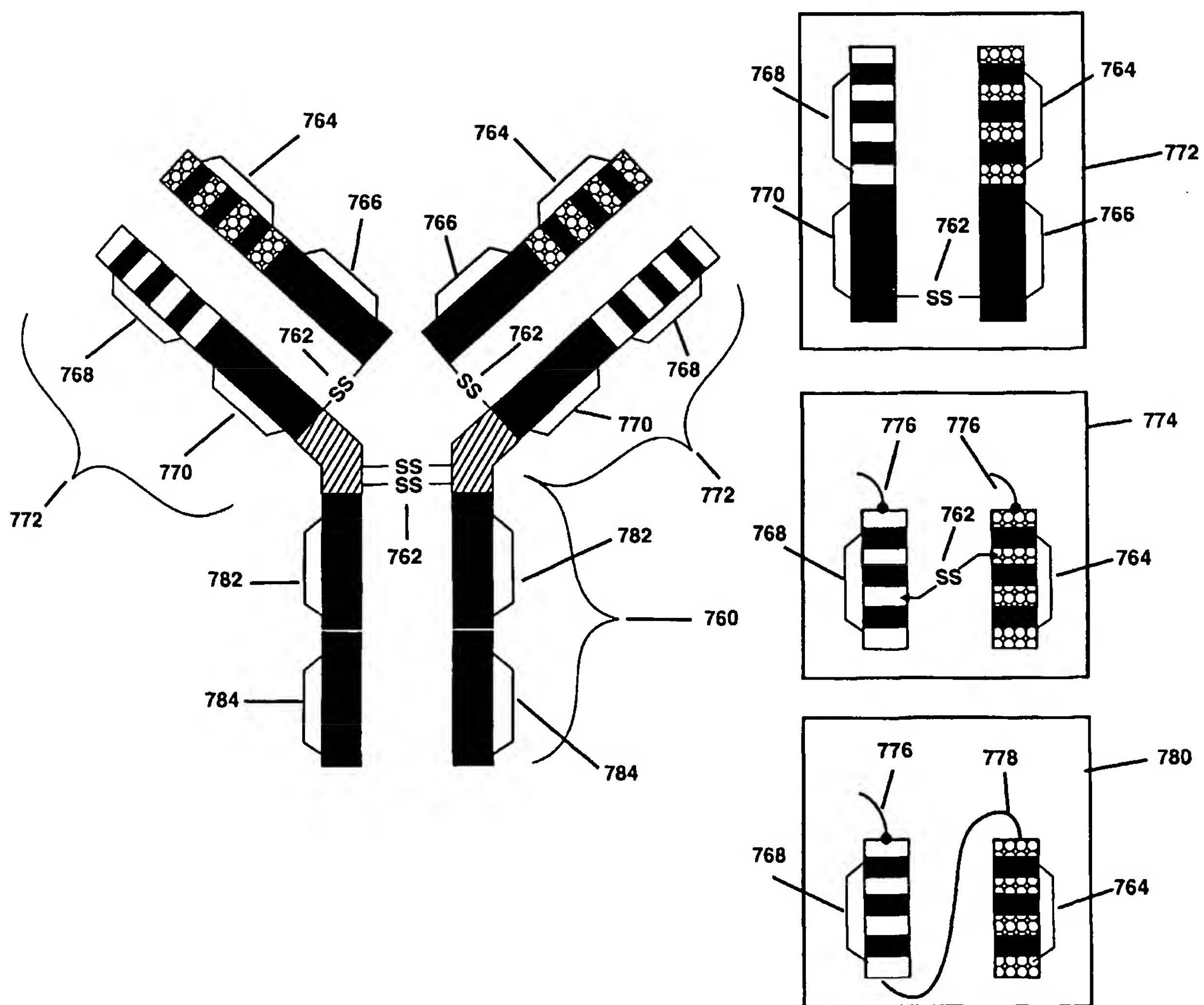


**Figure 5b.**

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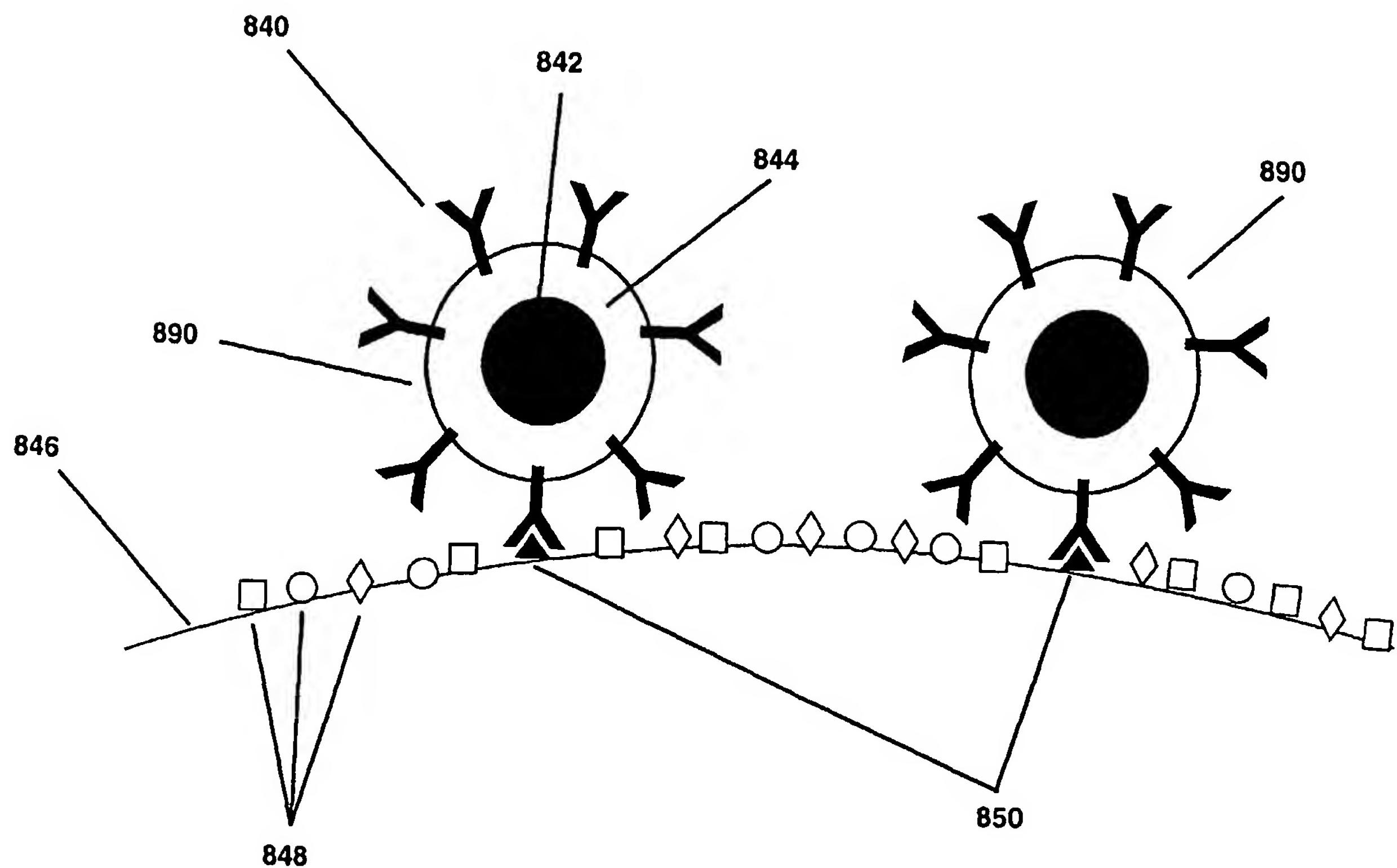


**Figure 6.**

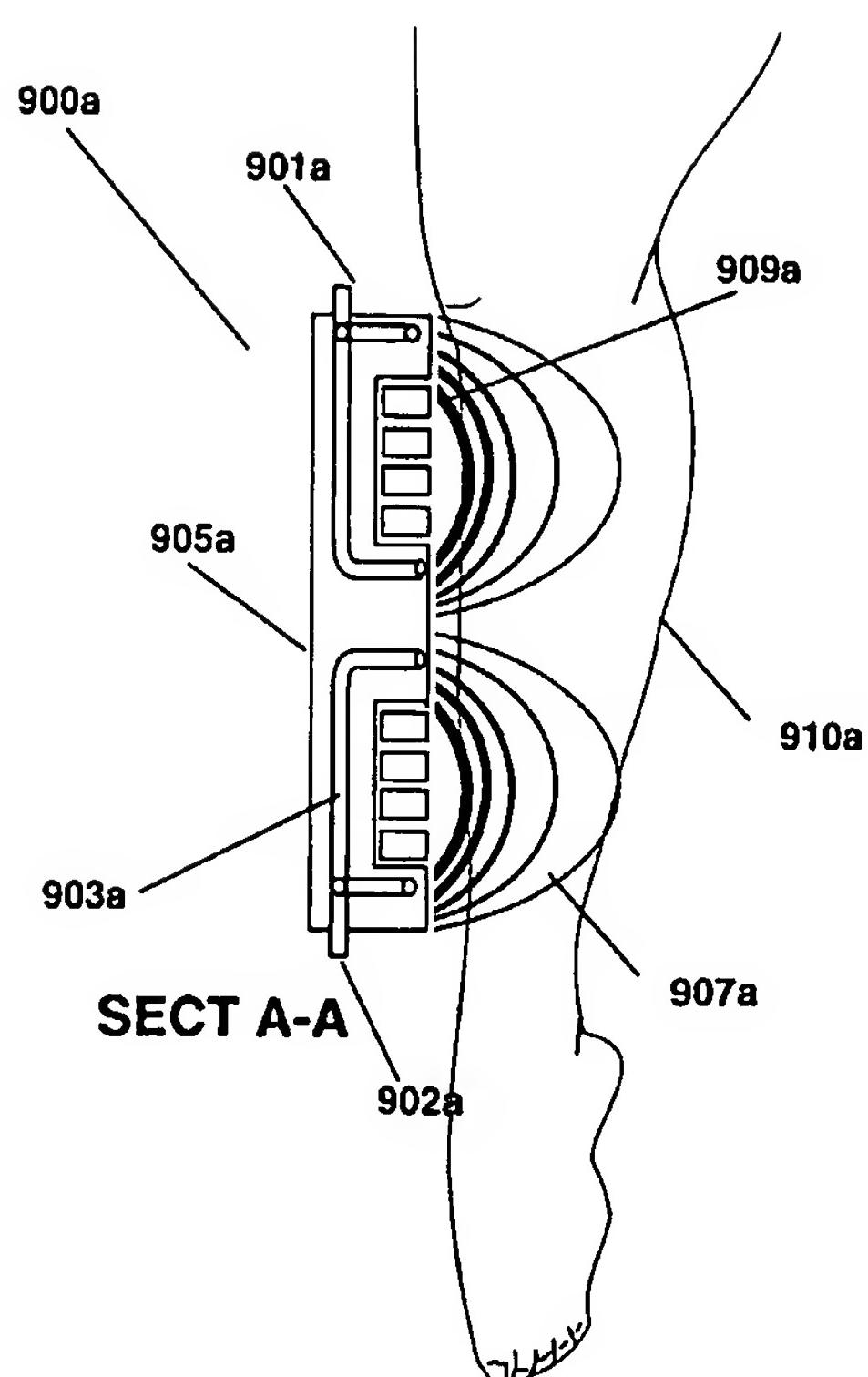


**Figure 7.**

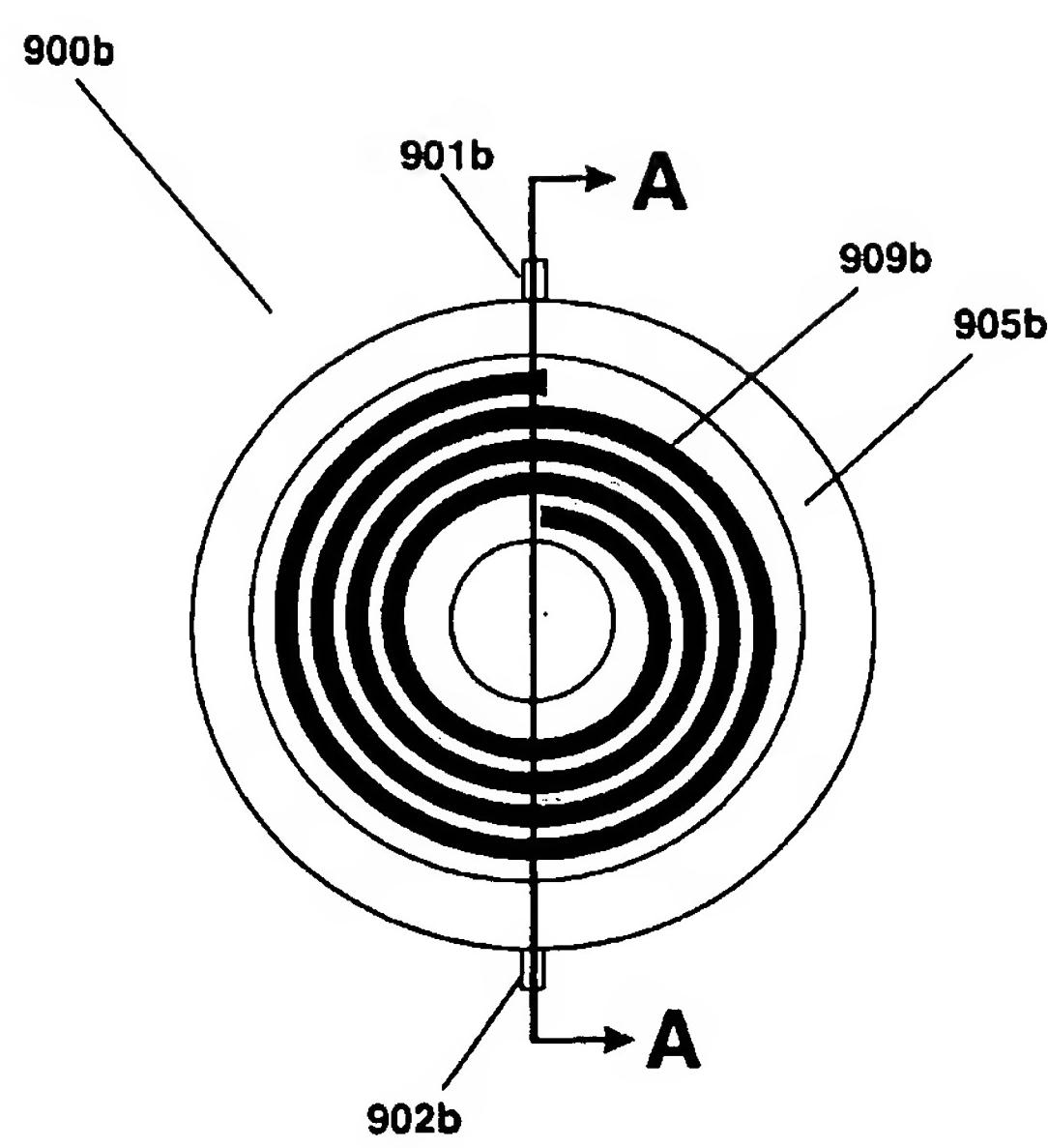
7/8



**Figure 8.**



**Figure 9a.**



**Figure 9b.**

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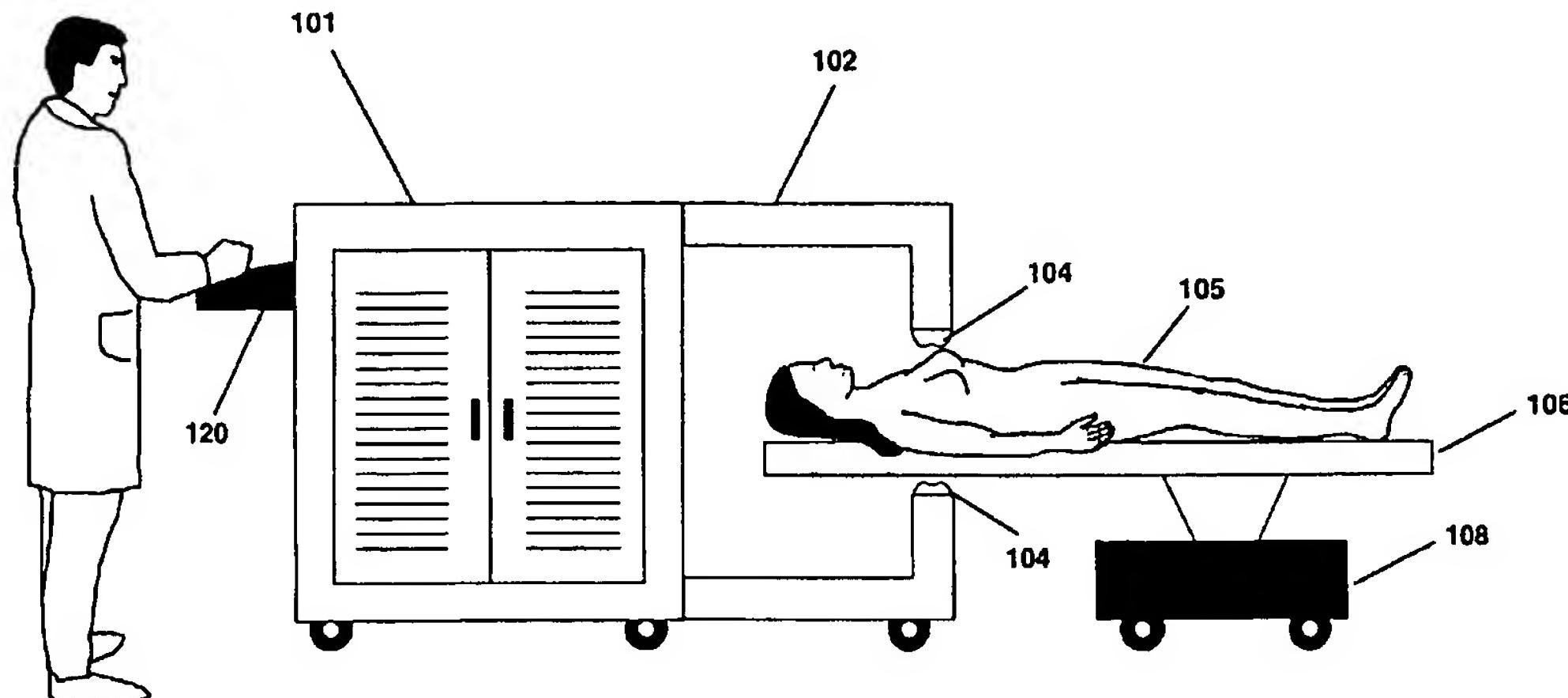
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[Continued on next page]

(54) Title: THERMOTHERAPY VIA TARGETED DELIVERY OF NANOSCALE MAGNETIC PARTICLES



**WO 03/022360 A3**



(57) **Abstract:** Disclosed are therapeutic methods for the treatment of diseased, disease-causing, or undesirable tissue or material that involve the administration of a thermotherapeutic magnetic composition, which contains single-domain magnetic particles attached to a target-specific ligand, to a patient and the application of an alternating magnetic field to inductively heat the thermotherapeutic magnetic composition. Also disclosed are methods for administration of the thermotherapeutic magnetic composition. These therapeutic methods may be used where the predetermined target is associated with a disease, such as cancer, diseases of the immune system, pathogen-borne diseases, obesity, hormone-related diseases, Alzheimer's disease, disease precursor, and undesirable materials, such as undesirable adipose tissue, reactions to organ transplants, HIV infected cells, polypeptides receptors, blood clots, and low density lipoproteins.



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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT
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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 37721 A (UNIV NEW YORK) 31 May 2001 (2001-05-31)	71
A	page 4, line 14 -page 7, line 9; figures	72,73, 75,77, 79,80, 97,99, 104
A	US 4 545 368 A (RAND ROBERT W ET AL) 8 October 1985 (1985-10-08)	71,74, 76-82, 107,108, 110,118, 121
	column 4, line 67 -column 12, line 39; figures	
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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 300 750 A (EKSTRAND JOHN P ET AL) 5 April 1994 (1994-04-05) column 2, line 13 - line 68; figures ---	71,77-80
A	EP 0 333 381 A (METCAL INC) 20 September 1989 (1989-09-20)  page 2, line 39 -page 3, line 37 page 4, line 3 -page 6, line 48; figures ---	71,77, 78,102, 107-110, 113,118
A	EP 0 543 498 A (MATSUMI HIDETOSHI ; SATO TOMOYA (JP); RIKEN KK (JP)) 26 May 1993 (1993-05-26) page 3, column 3, line 3 -page 4, column 5, line 8; figures ---	71,72, 77,78, 80-83
X	WO 01 10501 A (FEUCHT PETER ; MFH HYPERTERMIESYSTEME GMBH (DE)) 15 February 2001 (2001-02-15) the whole document ---	107,108
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**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/US 02/23650**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: 1-70 because they relate to subject matter not required to be searched by this Authority, namely:  
**Rule 39.1(iv) PCT – Method for treatment of the human or animal body by therapy**
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 71-106

A magnetic material composition, comprising:

a particle having magnetic properties and forming a single magnetic domain,  
a biocompatible coating material for the particle; and  
a ligand selective to at least one disease material marker associated with disease material, the ligand being one of bound to an uncoated portion of the particle, bound to a coated portion of the particle, bound to the particle and partially covered by the coating and intercalated into the coating.

2. Claims: 107-121

A device for treating disease material by interacting with magnetic particles targeted to disease material in a patient, the device comprising:

a magnetic generator having a core defining at least part of a magnetic circuit, two poles of the core defining a gap therebetween, a magnetic field passing between two poles, the gap being of sufficient size to receive a portion of the patient containing the disease material; and  
a power supply coupled to provide energy to the magnetic generator so that the magnetic field passing between the two poles alternates at a frequency of about 1 kHz or more.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

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